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Virology | Review

Dengue and severe dengue

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SUMMARY Dengue is an acute mosquito-borne viral disease that is highly prevalent throughout the tropical world. The geographic footprint of the four dengue viruses (DENV-1 to -4) that cause this disease and their *Aedes* mosquito vector is expanding, extending into North America and Mediterranean Europe. Furthermore, although dengue has historically been a disease that disproportionately affects children, changing population demographics and increasing travel to and from the tropics have contributed to a growing incidence in adults. Dengue in adults, particularly older adults, brings fresh and complex challenges to case management. Although dengue is now a vaccine-preventable disease, the efficacy profiles of licensed vaccines as well as those in late-stage clinical development suggest that vaccination alone would not fully retard

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the global expansion of dengue. Other countermeasures, including antiviral drugs, will be needed. This paper reviews the molecular interplay underlying dengue pathogenesis, including from virological and immunological perspectives, which are foundational for developing antiviral therapies and new vaccines. It also reviews the hurdles facing antiviral development and discusses new insights on dengue immunity that can guide the deployment of imperfect vaccines to begin reversing the global burden of dengue.

KEYWORDS dengue, dengue virus (DENV), host factors, host response, adult dengue, therapeutics, immunity, vaccines

INTRODUCTION

Dengue is an *Aedes* mosquito-transmitted acute viral disease. It is caused by infection with any one of four related but genetically distinct dengue viruses (DENV-1 to -4), belonging to the *Orthoflavivirus* genus in the *Flaviviridae* family. An estimated 100 million people fall ill with dengue each year, some with life-threatening severe dengue, although actual incidence fluctuates from year to year (1, 2). Not only are large numbers of endemic dengue cases reported throughout the tropics and in parts of the subtropics anually (3), but dengue also causes cyclical epidemics. These epidemics occur once every 3–7 years, with places having higher transmission intensity experiencing more frequent epidemics (4, 5). Surges in dengue cases during epidemics can profoundly overwhelm healthcare resources, leading to bed shortages and delays in access to elective healthcare procedures (6). Thus, beyond the health and economic burden on those who acquire dengue each year, epidemics also impose a major negative impact on the health of many with unrelated diseases. Indeed, the World Health Organization (WHO) has listed dengue as one of the top 10 major health concerns of this century (7).

Until the past decade, dengue prevention has relied almost exclusively on suppressing the mosquito vector population, especially *Aedes aegypti* (8); no other interventions were available. As *Aedes aegypti* is highly domesticated and adapted to the urban environment, reducing the abundance of *Aedes* larval breeding habitats, such as pails, unused flowerpots, and other water-containing or trapping receptacles, is an effective approach to reducing dengue incidence (9, 10). Such an approach, also known as source reduction, can result in remarkable control of dengue, as exemplified by the *Aedes* control programs of Cuba and Singapore (11). The major problem with such programs, however, is that with reduced *Aedes aegypti* abundance and virus transmission, population immunity to DENV infection is also lowered. Consequently, even with a few *Aedes aegypti* mosquitoes, epidemics continue to occur, as exemplified by the experience of Singapore (8, 12).

Fortuitously, there are now additional approaches to controlling dengue besides source reduction. Dengue is now a vaccine-preventable disease (13). Moreover, introgression of *Wolbachia*, an endosymbiont that reduces vectorial capacity of *Aedes aegypti*, has also shown efficacy in preventing symptomatic dengue at levels comparable with vaccination (14). Indeed, emerging evidence suggests that combining *Wolbachia*-based vector control with vaccination may yield synergistic or additive effects (15), further enhancing population-level protection against dengue. However, variability in introgression rates and hence efficacy in dengue prevention can be expected due to local climatic and environmental factors (16). Other tools, including the use of *Wolbachia* for *Aedes aegypti* population suppression (17) and genetically modified mosquitoes that are refractory to DENV infection (18), are in various stages of development.

Unfortunately, despite the availability of new tools to control dengue, the global burden of dengue is likely to worsen (19). Multiple reasons contribute to this worsening trend. With continued transnational travel and trade, along with climate change, the geographic distribution of dengue will continue to expand both northwards and southwards. More places will become hyperendemic for dengue, where all four DENVs circulate at any given time, making sequential infections even more likely (20, 21). Changing demographics of dengue patients, which is happening in many places, have

now shifted dengue from a disease that disproportionately affects children to one that increasingly afflicts adults and older adults (22). Such a changing geographic distribution of dengue and patient demographics calls for new countermeasures to reverse the growing global dengue burden and reduce the rate of preventable morbidity and mortality. This review is thus focused on reviewing the knowledge on DENVs, dengue pathogenesis, and immune mediators of protection that are foundational for tackling new challenges in case management, as well as therapeutic development and vaccine application.

DENV AND DENV INFECTION

DENVs are enveloped, single-stranded, positive-sense RNA viruses. Each of the four DENVs has a genome that is approximately 11 kb in length. An estimated 70% of the nucleotide sequence is shared, with further genetic diversity within each of the four DENVs (23); each of the four DENVs can be further subdivided based on full-length genome sequences into genotypes, and each genotype further classified into major and minor lineages (23). The open reading frame (ORF), which encodes 10 proteins, is flanked by 5′ and 3′ untranslated regions (UTRs) (Fig. 1). Both the 5′ and 3′ UTRs contain nucleotide sequences that form secondary RNA structures through base pairing. These RNA structures are highly conserved as they are necessary for virus replication (24–27). Furthermore, the 5′ and 3′UTRs also contain sequences that base pair to cyclize the DENV genome for replication (28–30). The structures within the 5′ and 3′UTRs, along with the function of these structures, have been reviewed elsewhere (31).

Besides the secondary RNA structures in the 5′ and 3′UTRs, base pairing also occurs in the other parts of the DENV genome, including within the ORF (32, 33). Secondary RNA structures within the ORF are less well characterized, although some appear necessary for viability of DENVs; synonymous substitutions that potentially disrupt these RNA-RNA interactions can reduce infection (33). Moreover, several of these secondary RNA structures were found only in extracellular DENV particles. The disruption of these secondary RNA structures through site-directed synonymous substitutions suggests that beyond virus-host interactions, these secondary RNA structures in the ORF may be important for packaging of the RNA for encapsulation (33).

The ORF is translated in the host cytoplasm as one single polypeptide. This polypeptide is then co- and post-translationally cleaved into the individual proteins by host and viral proteases (34). Of these 10 proteins, three are structural: capsid (C), pre-membrane (prM), and envelope (E). The C protein forms dimers and encapsulates the newly formed RNA genome (35, 36), which it releases upon infection of new susceptible cells through ubiquitylation in the cytoplasm (37). It is then enveloped by lipid membranes derived from the endoplasmic reticulum (ER), with the prM and E proteins embedded. The prM protein acts as a chaperone to prevent premature fusion of the E protein with ER and Golgi membranes during egress; it is further cleaved into the pr peptide and membrane (M) protein (38). The E protein is organized as a dimer on the lipid membrane of virions, which trimerizes in an acidic pH environment to enable fusion with the endocytic membrane upon infection (39).

All of the remaining non-structural (NS) proteins are involved in forming the RNA replication complex and avoiding host innate immune responses. Detailed review on the structure of the complex, as well as the functions of the individual NS proteins in immune evasion, can be found elsewhere (40–42).

UPDATES ON DENGUE PATHOGENESIS

DENV infection

DENV enters the target cells, mostly of myeloid lineages in human infections (43), either through binding cognate plasma membrane receptors, of which there are several candidates, or through activating fragment crystallizable gamma receptors (FcγR) (44). The latter occurs when antibodies that develop after a primary infection bind

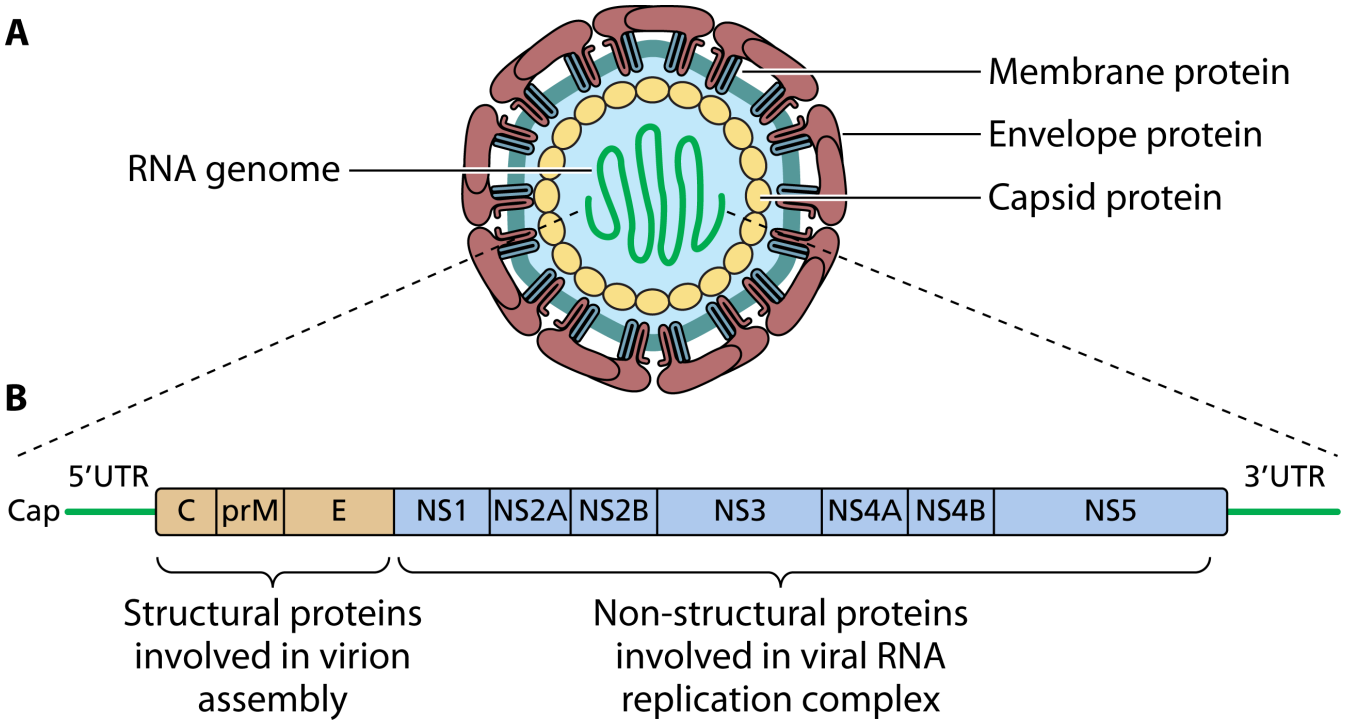


FIG 1 Schematic representation of DENV. (A) Cross-sectional representation of the virion and the encapsulated DENV positive-sense RNA genome. (B) Schematic representation of the DENV RNA genome. Both the 5′ and 3′UTRs are highly structured and here, for the sake of simplicity, are represented by straight lines. The ORF of the genome consists of 10 genes, three of which encode structural proteins, whereas the remaining seven encode non-structural proteins.

a heterologous DENV, causing a secondary infection and facilitating DENV-immune complex uptake by myeloid-derived cells via activating FcγR (45). Acidification of the endosome or phagosome leads to trimerization of the E protein that then initiates fusion with endosomal/phagosomal membranes (39). The nucleocapsid is then released into the cytoplasm. Viral RNA is released from the capsid upon ubiquitylation (37), whereupon translation of the viral proteome, replication of the viral RNA, and assembly of new virions occur on the ER (Fig. 2). Newly formed DENV would then mature, where the pr peptide is cleaved from the M protein through host furin activity (46), and egress via the trans-Golgi network; the pr peptide separates from the virion in the extracellular space (47) (Fig. 2). However, DENV maturation is not completely efficient. Newly formed DENVs contain a mix of mature, partially mature, and immature viral particles—the latter two categories of virions could have immune evasion properties (reviewed in reference 48). Besides newly formed virions, infected cells also secrete the viral NS1 protein (Fig. 2), the function of which may have implications for disease pathogenesis, which is discussed in later sections of this review. Although the structure of the individual NS1 and, hence, how NS1 dimers form in the ER have been resolved (49), the structure of the secreted NS1 (sNS1) protein is still a subject of debate; different cryo-electron microscopy studies have shown them to be either dimeric (50), tetrameric, or hexameric (51).

DENV-host interactions

Given the very limited proteins encoded within the DENV genome, DENV necessarily interacts with host cell components to supplement its limited proteome to complete its lifecycle in infected cells. These interactions involve not only host proteins (52, 53) but also host RNA and lipids (41). The requirement for such interactions, as well as the molecules with which DENVs necessarily interact, can be gleaned from functional genomics studies. These studies using knockdown or knockout approaches have identified host genes, which when disrupted, reduced the number of DENV progeny

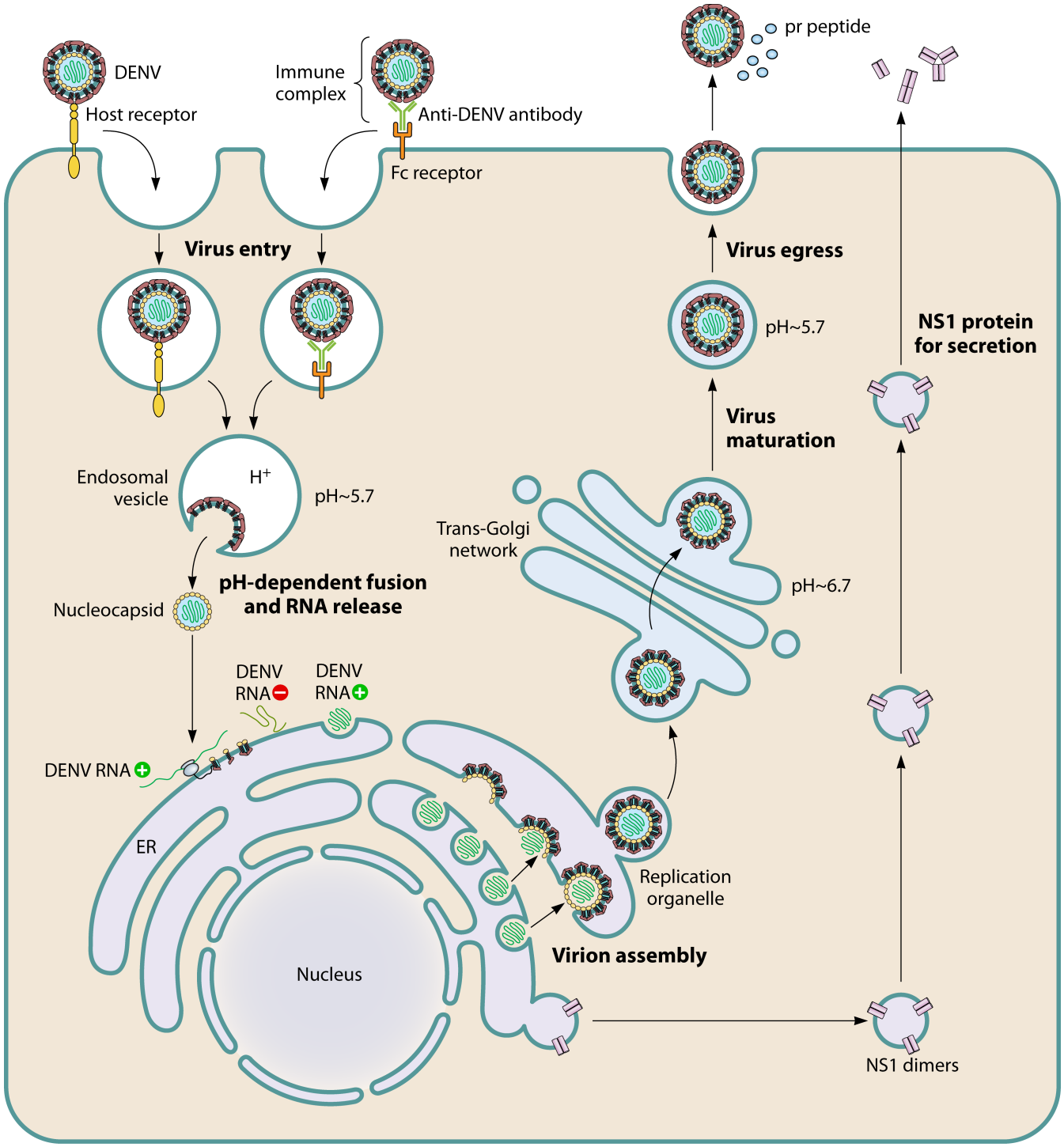


FIG 2 Lifecycle of DENV in a mammalian cell. The lifecycle is represented from left to right in an anticlockwise direction. DENV infects the cells through either receptor-mediated endocytosis or FcγR-mediated uptake. Fusion of the viral membrane via conformational changes in the E protein releases the capsid and RNA into the cytoplasm. RNA is released from the capsid upon ubiquitylation of the capsid, whereupon the RNA undergoes translation and replication in highly reorganized ER membranes or replication organelles. Assembly of new virions occurs with the replication organelles before egress via the trans-Golgi pathway. Besides new virions, infected cells also secrete the NS1 protein into the extracellular space.

produced during infection (54–56). These genes thus encode molecules essential for successful DENV replication and are commonly referred to as host factors or host proviral factors. Conversely, genes, which led to increased DENV replication when disrupted,

encode host-restrictive or antiviral factors. Thus, the efficiency of DENV infection is influenced by the availability of proviral factors and the suppression of restrictive factors.

Many of the host proviral factors are proteins involved in ER function (53). This is perhaps not surprising since DENV RNA replication, transcription, and translation, as well as progeny assembly, all happen on ER membranes (57). Imaging studies of DENV-infected cells have found extensive rearrangements of ER membranes into replication organelles, which facilitate DENV RNA genome synthesis, viral protein translation, and virion assembly (57, 58). Besides viral replication, the reorganization of the ER in DENV-infected cells may also serve an immune evasion function. As the mitochondria are closely linked to the ER and play important roles in both innate and adaptive immune responses, formation of replication organelles could also disrupt mitochondrial function to dampen innate immune response within infected cells (59). More details on DENV replication organelles can be found elsewhere (60, 61).

Despite the genetic differences between the four DENVs and other orthoflaviviruses, functional genomics studies have revealed common proteins critical for successful infection (62). For example, host proviral factors that have been universally found among the top hits in CRISPR/Cas9-based screens on DENVs (56, 62–64), as identified using a recently developed search system (65), are all ER membrane proteins (Fig. 3). Finally, infection requires energy. Consequently, DENV also requires interaction with proteins that regulate mitochondrial function (66) and metabolism (64).

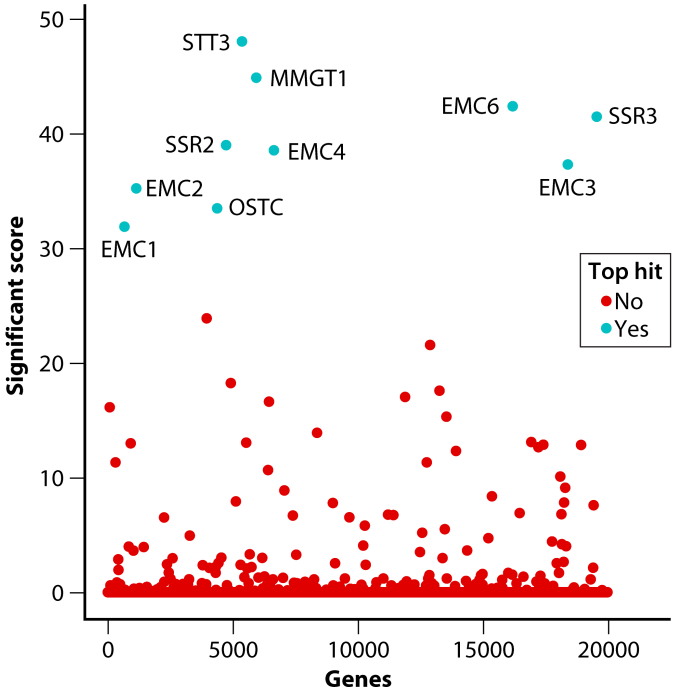
Host-restrictive factors are mostly but not exclusively part of the innate immune system (reviewed in reference 67). A well-known group of host-restrictive factors is those involved in the type-I interferon (IFN) response. Type-I IFN can be induced by host pattern recognition receptors (PRR), which can detect both viral proteins as well as viral RNA (68, 69). Plasma membrane and cytoplasmic sensors, such as toll-like receptors (TLR) and retinoic acid-inducible gene-I (RIG-I), can sense viral proteins, as well as viral RNA during infection and upon uncoating of viral RNA (reviewed in reference 68). Upon activation of any of these PRRs by DENVs in the course of infection, a cascade of signaling factors would be initiated, resulting in the expression of type-I IFN. Secreted type-I IFN then functions in an autocrine or paracrine manner to induce interferon-stimulated genes (ISGs), such as interferon-induced transmembrane protein 1 (IFITM1), to inhibit DENV infection (70). *In vitro* studies have shown that the paracrine activity of type-I IFN is more effective than the autocrine functions; addition of exogenous type-I IFN to cells after DENV infection is not as effective in restricting DENV infection as addition before infection (71, 72). ISGs are thus more effective at restricting the dissemination of DENV infection systemically than controlling infection within infected cells.

Besides host proteins, host RNA also plays an important role in supporting or restricting DENV infection. Among the host RNAs are microRNA (miRNA). MiRNAs are small, single-stranded, non-coding RNAs that regulate gene expression at the post-transcriptional level. MiRNAs can both support DENV infection or mediate antiviral functions. Several *in vitro* studies have suggested several miRNAs that support DENV infection (73, 74). On the other hand, miRNAs that were found to be differentially expressed in dengue seropositive compared with seronegative individuals living in a highly endemic area, showed antiviral functions (75). Indeed, an unbiased RNA-RNA interactome study has shown that DENV RNA interacts with host RNA, which may either be pro-viral or antiviral in function (76). How these miRNAs support or antagonize DENV replication and are regulated during infection remains to be determined.

Virus genetics and infection

Given the interactions between viral proteins and RNA with host factors and their avoidance of host innate immune sensors, genetic differences could have major implications on infection outcome and pathogenesis. Broadly speaking, DENV-1, -2, and -3 are responsible for much of the global dengue burden; DENV-4 has been associated with fewer outbreaks than the other three DENVs (3). Furthermore, genetic differences within each of the four DENVs can also impact dengue epidemiology and infection

A



B

Gene	Protein	Cellular compartment
STT3A	STT3 oligosaccharyltransferase complex catalytic subunit A	ER membrane
MMGT1	Membrane magnesium transporter 1	ER membrane
EMC6	ER membrane protein complex subunit 6	ER membrane
SSR3	Signal sequence receptor subunit 3	ER membrane
SSR2	Signal sequence receptor subunit 3	ER membrane
EMC4	ER membrane protein complex subunit 4	ER membrane
EMC3	ER membrane protein complex subunit 3	ER membrane
EMC2	ER membrane protein complex subunit 2	ER membrane
OSTC	Oligosaccharyltransferase complex non-catalytic subunit	ER membrane
EMC1	ER membrane protein complex subunit 1	ER membrane

FIG 3 Host factors shared by all four DENVs. (A) Genes (green dots) identified from published CRISPR-Cas9 screens as required host factors of all four DENVs. Knockout of these genes abrogated DENV infection. (B) Proteins encoded by these genes and the cellular compartment in which these proteins are commonly found. Data from reference 65.

outcome. Indeed, the influence of viral genetics on pathogenesis can be gleaned from the successful demonstration of safety in the tens of thousands of volunteers who have participated in clinical trials of live-attenuated dengue vaccines. Among the dengue vaccines that have either been licensed or completed phase III clinical trials, TAK-003 contains the attenuated DENV-2 strain, PDK53, whereas TV003 contains attenuated DENV-1, -3, and -4. The other components of these two vaccines are chimeric viruses and would thus not be considered in this discussion.

Compared with its wild-type parent, the DENV-2 16681 strain that was isolated from a dengue patient, PDK53, generated by 53 passages in primary dog kidney cells, contains one nucleotide substitution in the 5′UTR and five non-synonymous substitutions in the ORF (77). Among the five amino acid substitutions in PDK53 ORF is the glycine to aspartic acid substitution on position 53 of the NS1 protein (NS1 G53D). This substitution was shown to compromise the interaction between NS1 and the oligosaccharide transferase complex (OST). Reduced OST complex enzymatic activity lowered NS1 protein glycosylation, resulting in misfolding that attenuates infection in both *Aedes aegypti* and human cells (78). As few as one mutation could thus have a major impact on DENV pathogenesis.

Besides vaccine strains, epidemiological observations also suggest an association between DENV genetics and dengue epidemics (79, 80). Indeed, the association between the emergence of new viral phylogenetic clades and epidemic dengue has been reported in several locations globally (81–88). However, mechanistic explanations for how genetic differences alter the epidemiological fitness of DENV have, with a few exceptions, mostly remained undefined (reviewed in reference 89). The following examples show how as few as one mutation could have a major impact on the epidemiological phenotype of DENV.

Dengue epidemiology in the South Pacific in the 1970s and the dengue epidemic in Puerto Rico in 1994 are two such examples. An American genotype of DENV-2 emerged in French Polynesia in the early 1970s, which then spread rapidly to the neighboring

islands, including New Caledonia, Niue, and Fiji. In all these islands, the epidemics were severe, with many developing the more severe form of dengue (90). However, no outbreak was detected in Tonga when this DENV-2 emerged in 1974; only mild febrile cases were encountered (91). Phylogenetically, the DENV-2 that emerged in Tonga segregated from those isolated from the other South Pacific islands (86). Among the genetic differences, a recent investigation was able to attribute the epidemiological phenotype to a single nucleotide substitution in the prM protein, which compromised viral genome translation, likely through codon usage bias. This mutation, however, encoded a histidine-to-arginine substitution in position 86 of the prM protein, which improved the infection of *Aedes aegypti* midgut. The arginine residue could maintain the positive charge despite the basic pH in the mosquito midgut to enable DENV-2 attachment to receptors with negative charge for viral entry (92). Thus, despite the apparent reduced fitness in human cells, the DENV-2 that emerged in Tonga was able to sustain transmission for several years (91).

Conversely, an Asian-American genotype of DENV-2 that emerged in Puerto Rico in 1982 did not cause an outbreak until 1994. Autochthonous transmission enabled the virus to accrue substitutions in its genome. In particular, five amino acid substitutions in the NS5 protein (93) and three nucleotide substitutions in the 3′UTR (94) enabled the virus to generate more subgenomic RNA relative to its genomic RNA. Subgenomic RNA, also known as subgenomic flavivirus RNA (sfRNA), is a product of host exonuclease 1 (XRN1), which digests viral RNA from a 5′-to-3′ direction, until the enzyme stalls due to the secondary and pseudoknot RNA structures in the 3′UTR (95, 96). Besides stalling XRN1, the highly structured sfRNA also interacts with many other host factors and is required for pathogenicity (97, 98). Mutations in the NS5 resulted in a slower rate of genomic RNA replication, allowing the rate-limiting XRN1 to generate relatively more sfRNA compared with gRNA (93). Moreover, the three nucleotide substitutions in the 3′UTR increased the binding of sfRNA to host TRIM25 protein. TRIM25 is an E3 ligase that ubiquitylates the cytoplasmic RNA sensor, RIG-I, to amplify and sustain RIG-I-mediated signaling for type-I IFN induction. Inhibition of TRIM25 thus compromised type-I IFN response to infection, thereby allowing a greater level of systemic dissemination (94).

Given the large differences in clinical and epidemiological phenotypes from as few as a single mutation in the DENV genome, it is rather unfortunate and even alarming that functional genomics studies have only compared host factor requirement differences between the four DENVs. Only one study has performed a CRISPR/Cas9 screen to show how a single mutation altered host factor requirements. This study found that a single amino acid substitution in the NS2B protein, which reduced NS2B interaction with the signaling intermediate molecule STING, necessitated additional host factors–EID3, NEK5, and RAD21–that repair damaged DNA, increased mitochondrial activity, and hence DNA damage needed to be controlled for successful infection (63). Functional genomics of closely related DENV strains with interesting clinical and epidemiological phenotypes could yield useful insights into virus-host interactions critical for pathogenesis and transmission.

Antibody-enhanced infection

The four DENVs share approximately 60%–70% of their structural antigens (99). Infection with one DENV would therefore not only induce type-specific (homotypic) antibodies, which recognize and bind to epitopes on the E protein unique to the homologous DENV and typically strongly neutralize that DENV, but also induce cross-reactive (heterotypic) antibodies that bind to conserved E protein epitopes shared among all four DENVs with varying degrees of neutralizing activity (100, 101). Following infection with any DENV, the adaptive immune response that develops provides long-term immunity to the homologous virus, although breakthrough re-infection with the same DENV type has been reported (102, 103). Cross-protection from heterologous DENV infection is temporary: human challenge studies by Albert Sabin suggested that this cross-protection lasts approximately 3 months (104, 105), whereas epidemiological studies estimate a longer

duration of approximately 2 years (106, 107). With hyperendemicity, sequential infections with heterologous DENVs have become prevalent (20).

Not only is cross-protective immunity short-lived, but adaptive immune response to one DENV appears to increase the risk of severe secondary infection with a heterologous DENV (82). Although severe dengue can occur in both primary and post-primary infections (108, 109), the risk appears to be the highest in secondary heterologous DENV infection (110, 111). This increased risk of severe dengue is widely accepted to be mediated by antibody-dependent enhancement of DENV infection (ADE). First postulated by Halstead (112, 113), ADE occurs when cross-reactive antibodies or sub-neutralizing levels of IgG antibodies bind heterologous DENV to facilitate virus entry through activating FcγRs that are expressed on myeloid-derived cells such as monocytes, macrophages, and dendritic cells. Furthermore, FcγR-mediated virus entry appears more efficient than cognate receptor-mediated endocytosis (114), although non-antibody-mediated infection could be mediated by more than one receptor (115). Perhaps the strongest evidence for ADE is the association between severe DENV infection in infants at a time when maternal antibodies wane to sub-neutralizing levels (116–118). As transplacental transfer of maternal immunoglobulin is limited to IgG antibodies, the increased risk of severe dengue provides an opportunity to understand ADE clinically (119).

Although most studies on ADE have focused on anti-DENV IgG antibodies that interact with FcγRs, it is worth noting at this point that the different classes of antibodies can compete with IgG for binding to DENV and nuance infection outcome. Before class-switching from IgM to IgG, a single pentavalent IgM can concurrently bind 10 antigen-binding sites, compared with just two with IgG, resulting in greater virus neutralization potency (120). Monomeric IgA antibodies also develop following infection (and likely vaccination) to compete with IgG for DENV. Furthermore, the interaction of the IgA-DENV complex with FcαR does not result in ADE (121, 122). However, both IgM and IgA antibody titers decay after recovery from infection and do not persist at levels comparable with IgG.

Besides competition from different classes of antibodies, the lack of universality in increased risk of severe dengue in infants from waning maternal anti-dengue IgG titers could be because ADE requires more than just FcγR-mediated entry. Activating FcγR signals through the immunoreceptor tyrosine-based activation motif (ITAM), which activates the spleen tyrosine kinase (SYK) and signal transducer and activator of transcription-1 (STAT-1) (Fig. 4). Activated STAT-1 would then induce the expression of interferon-stimulated genes (ISGs) to effect antiviral functions (123). For enhanced DENV infection, DENV thus has to avoid activating this signaling pathway through, at least in part, binding the leukocyte immunoglobulin-like receptor B1 (LILRB1) (114, 124) (Fig. 4). Such altered signaling despite activating FcγR-mediated entry could alter not only cellular intrinsic response to infection (125–127) but also trafficking of DENVs to cellular compartments more suited for replication (128).

However, not all secondary infections have resulted in increased risk of severe dengue. Prospective cohort studies have shown that the risk appears to rise and ebb within a specific range of antibody titers (101, 129). Indeed, instead of activating FcγR, high titers of cross-reactive antibodies could aggregate DENVs to bind and co-ligate the inhibitor FcγRIIB (or CD32B), which has a cytoplasmic immunoreceptor tyrosine-based inhibitory motif (ITIM) (130), the signaling of which inhibits DENV entry (131) (Fig. 4). Furthermore, the glycosylated state of IgG antibodies could further influence the type of activating FcγR that DENV-IgG complex engages. Several studies have shown that afucosylated anti-DENV IgG antibodies selectively bind FcγRIII (or CD16), which is more highly expressed on non-classical (CD14+ CD16++) monocytes, which are more pro-inflammatory in response to infection (132–134). Finally, even ADE from waning titers of maternal antibodies may be influenced by the type of DENV. Although infection with any of the four DENVs can cause severe disease, infection with DENV-2 (116, 118), but not DENV-3 (135), appears, at least in infants, to be associated with a high risk

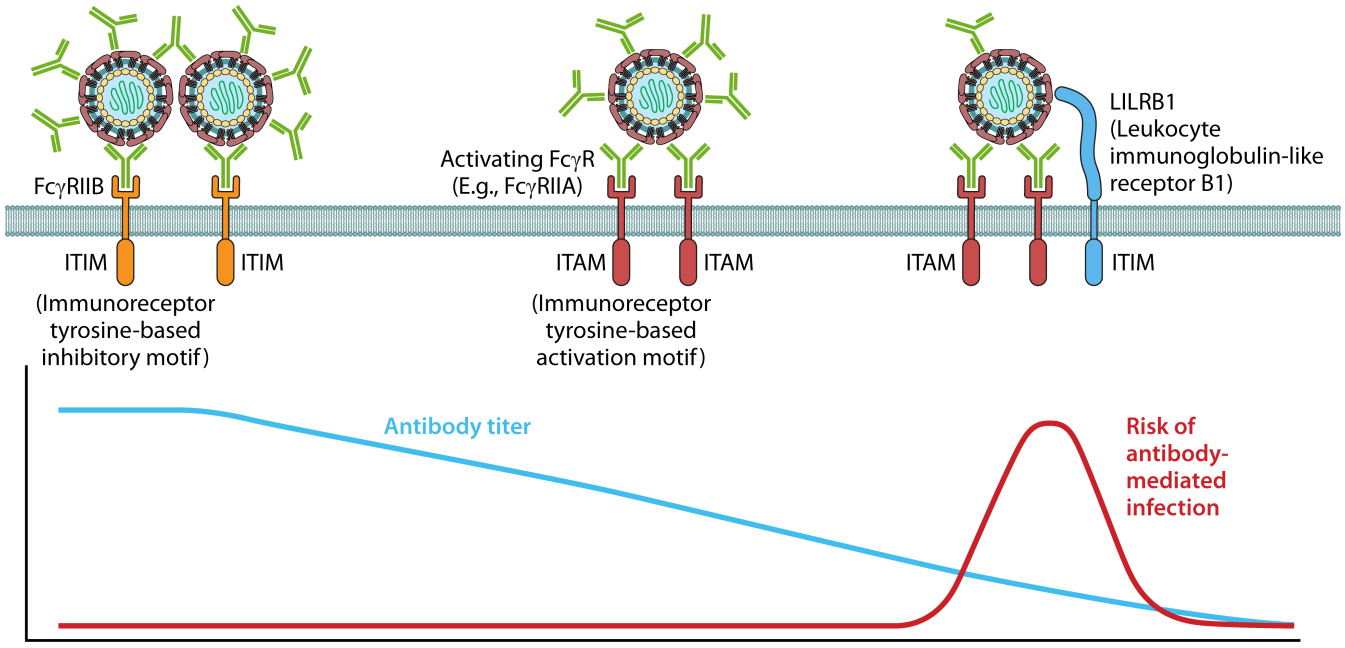


FIG 4 Ratio of IgG:DENV affects the outcome of interactions with myeloid-derived cells. Antibody-dependent enhancement of infection requires an optimal IgG:DENV ratio. High IgG titers can aggregate DENV to co-ligate the inhibitory FcγRIIB, the signaling of which prevents DENV entry. At a lower IgG:DENV ratio but at stoichiometry that blocks fusion, DENV infection would remain neutralized. Furthermore, activating FcγR signaling would induce STAT1 phosphorylation and ISG expression to inhibit DENV infection. To avoid STAT1 activation, DENV binds LILRB1 to inhibit early ISG expression for enhanced infection.

of severe dengue. Field studies of both dengue in infants, as well as those in older children, have also found differences between the four DENVs in the rate of symptomatic primary and secondary infection (136). Furthermore, there are limited data, suggesting that the antigenic differences between the DENV responsible for primary and secondary infections may also influence secondary dengue outcome (137). Clearly, a detailed dissection of the nuances of ADE with each of the four DENVs is still much needed.

Besides the production of antibodies by long-lived plasmablasts, prior DENV infection also produces memory B cells that express anti-DENV B cell receptors (BCRs). Recent findings suggest that such BCRs could bind DENV and serve as entry receptors for memory B cell infection (138, 139). The significance of memory B cell infection, however, is not entirely clear at this stage. It is possible that infected memory B cells act as Trojan horses when they migrate into lymphoid tissues to disseminate infection of myeloid-derived cells (138).

Immunopathogenesis

Multiple factors converge on downstream pathogenic pro-inflammatory responses and plasma leakage (Fig. 5). DENV infection burden, as measured through viremia levels in dengue patients, has shown positive correlation with disease severity (140, 141). Greater levels of DENV infection would result in more viral antigenic burden that exacerbates the pro-inflammatory response, ultimately resulting in increased vascular permeability; excessive vascular leakage could lead to shock, hemorrhage, and end-organ failure (142). One such DENV antigen that has been proposed to exacerbate inflammation is the secreted NS1 (sNS1) protein (143) (Fig. 2). In experimental mouse models, sNS1 has been shown to cause vascular leakage by directly causing endothelial dysfunction (144) and as a driver for the release of proinflammatory cytokines from immune cells (145, 146). Treating these mouse models with anti-NS1 antibodies (147, 148) or vaccinating the animals with NS1 antigen (149) protected against lethal DENV infection. However, the evidence from clinical studies is less clear. Studies that favor (150) or disfavor (151) the sNS1-driven pathogenesis hypothesis have been reported. Plasma sNS1 is more likely

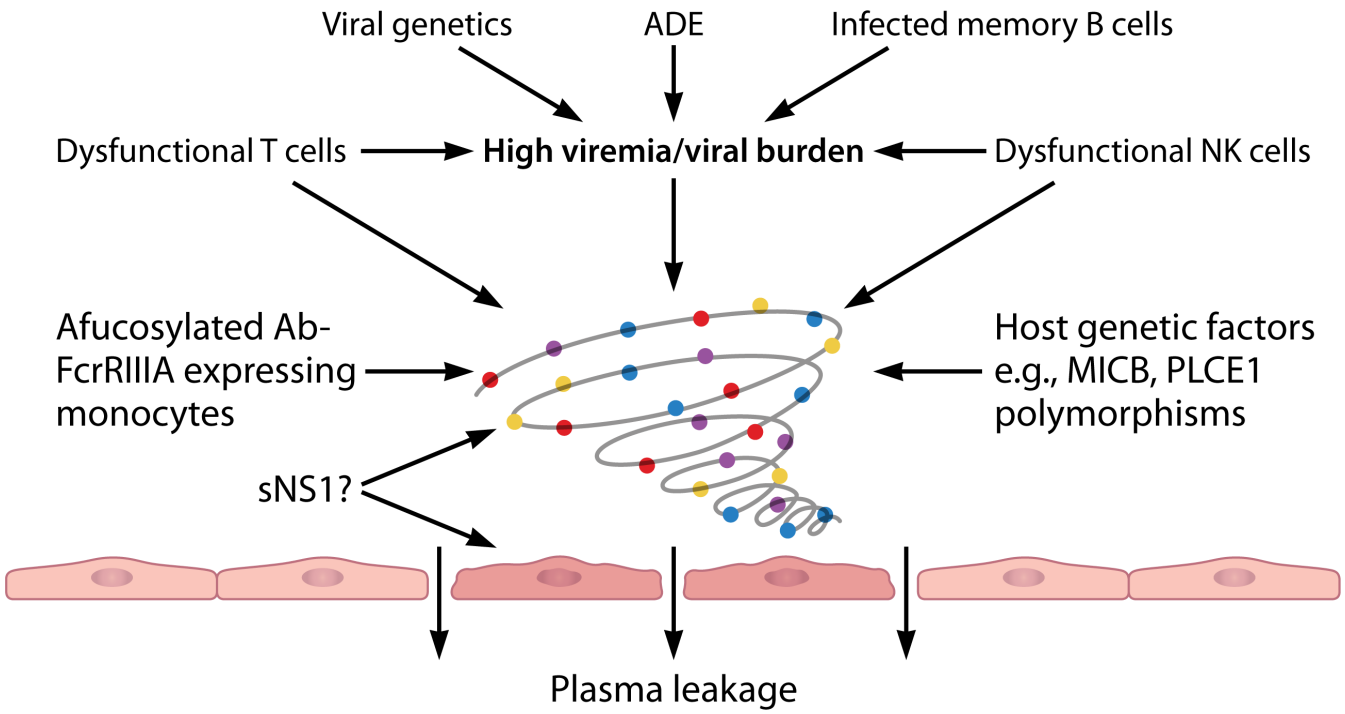


FIG 5 Contributors to severe plasma leakage. A summary of the factors that have been shown to contribute to increased viral burden (both increased DENV infection/replication or reduced DENV clearance), as routinely measured by viremia. Increased viral burden stimulates production of pro-inflammatory cytokines to levels that damage endothelial cell integrity, resulting in plasma leakage. ADE: Antibody-dependent enhancement.

to be undetectable in patients with secondary compared to primary DENV infection (152). Furthermore, plasma NS1 can be detected in primary dengue patients well into convalescence (153). The disconnect between animal models and clinical observations on the role of NS1 will require further investigations.

Regardless of which DENV protein, RNA, or host factors such as mitochondrial DNA activate PRRs in infected cells, the signaling cascade from high viremia or viral burden results in high levels of plasma pro-inflammatory cytokines, or cytokine storm (reviewed in reference 154). Cells that express such cytokines are the myeloid-derived cells targeted by DENVs (138) and also other cells, including mast cells (155, 156) and neutrophils (157–160). The pro-inflammatory response activates endothelial cells, causing them to express adhesion molecules, reduce production of nitric oxide, and alter both structure and function of the glycocalyx (161–163). The hyperinflammatory states in patients with severe dengue could be further exacerbated by compromised cell-mediated immune functions against DENV infection (164, 165). Unbiased immune cell profiling has also shown an association between dysfunctional T cells and higher levels of plasmablasts in hospitalized compared with ambulatory dengue patients (166, 167). Finally, more recent studies in patients with severe dengue have independently shown that natural killer (NK) cell dysfunction could also underpin disease severity (138, 168). Indeed, gene expression in immune cells can potentially be a prognostic marker of severe dengue (169–171).

Host susceptibility factors for severe dengue

As multiple interactions and molecules involved in dengue pathogenesis, it is thus not surprising that host genetic polymorphisms are associated with dengue severity. Given the roles of ADE, dysfunctional T cells, and inflammation in dengue pathogenesis, polymorphisms in genes of activating FcγRIIA (172, 173), inflammatory and anti-inflammatory cytokines (174, 175), and human leukocyte antigens (HLA) (176, 177) have also shown association with dengue severity. Likewise, as metabolism regulates immune cell function (178, 179), polymorphisms in oxysterol binding protein-like 10 (*OSBPL10*)

and retinoid x receptor alpha (*RXRA*) genes may explain the reduced susceptibility of those of African descent to severe dengue (180). The only genome-wide association screen (GWAS) that has been published thus far, which compared over 3,500 severe dengue cases with almost 5,000 healthy volunteers, identified disease susceptibility loci in the MHC class I chain-related protein B (*MICB*) and phospholipase C epsilon 1 (*PLCE1*) genes (181). These GWAS findings were validated in a separate study in Thailand, where single-nucleotide polymorphisms in MICB and PLCE1 genes showed higher and lower risks for DSS, respectively (182). Remarkably, MICB is a receptor on NK cells and PLCε1 is involved in intracellular signaling, both of which are involved in dengue pathogenesis, as discussed above (Fig. 5).

CHANGING DENGUE EPIDEMIOLOGY

Worsening global dengue burden

Dengue is prevalent throughout the tropical world and rapidly encroaching into the subtropics (Fig. 6). Its geographic distribution has been driven by urbanization, global trade, and travel (183). These human activities have spread both DENVs and *Aedes* vectors. For instance, after the successful Pan American *Aedes* eradication program was halted in the 1960s, *Aedes aegypti* was reintroduced into the Americas as early as the 1970s; ensuing increase in trans-continental travel introduced DENVs back into cities of Latin America, causing repeated outbreaks (184). Furthermore, populations in urban centers around the world are also growing; more than half of the world's population now lives in cities, where *Aedes aegypti* thrives (11). Many of these cities lack proper public health programs, such as providing a reliable water supply and reducing the number of domestic water containers that serve as *Aedes* larval habitats and daily garbage disposal, to prevent DENV transmission (19).

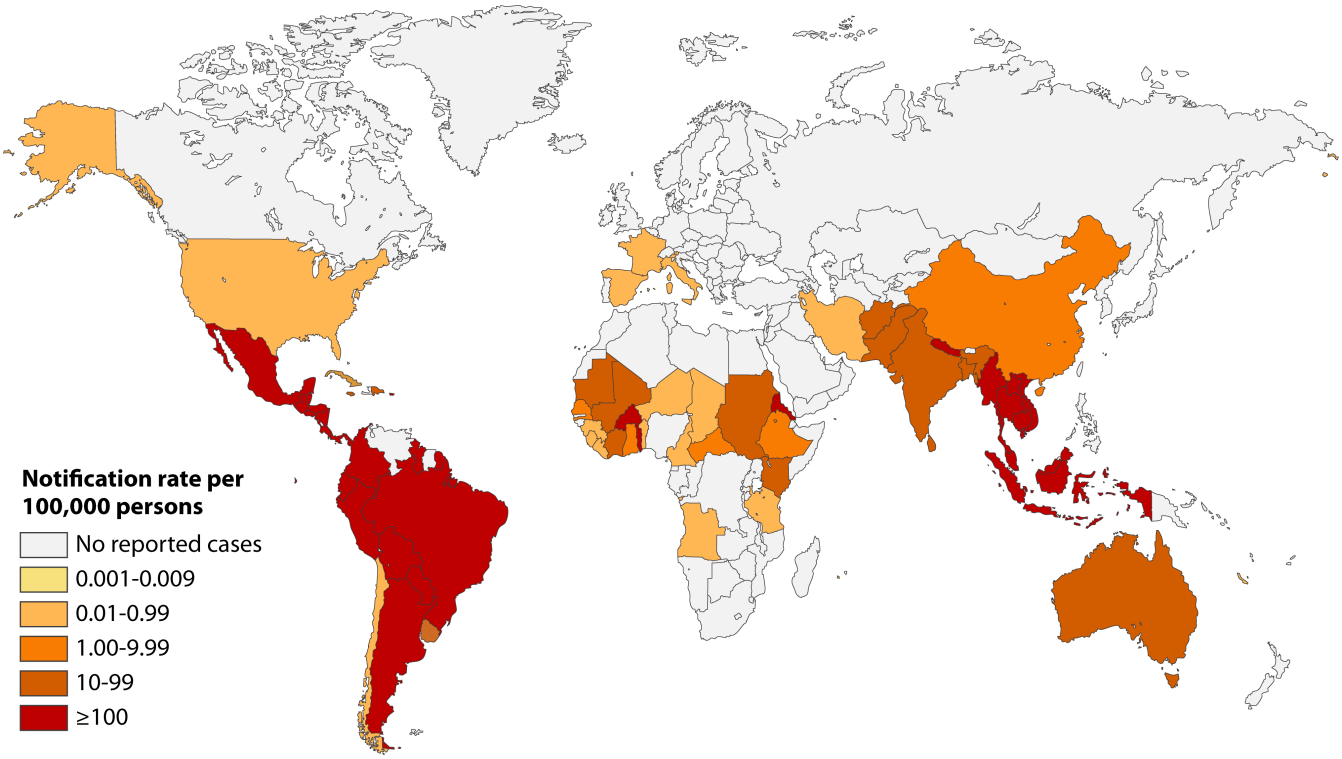


FIG 6 Global distribution of dengue cases. The map is based on the data collected between June 2024 and May 2025. Source: European Centre for Disease Prevention and Control (https://www.ecdc.europa.eu/en/publications-data/twelve-month-dengue-virus-disease-case-notification-rate-100-000-population-june).

Compounding the increased international travel and trade activities, as well as urbanization, is climate change–a challenge of the 21st century. It is reshaping the environment and human activities in profound ways. The impact of rising temperatures and sea levels, as well as less predictable seasonal weather patterns with more frequent extreme weather events, such as El Niño and La Niña events, not only impacts the environment but also dengue. For instance, meteorological phenomena such as South Indian Ocean oscillations have been shown to predict long-term global trends (185). With climate change, dengue is expected to increase even further in the coming years (186). Indeed, the year 2024 has witnessed the highest ever dengue incidence globally (187). Besides worsening dengue in endemic regions, climate change is also contributing to the expansion of dengue outbreaks into new geographical regions, both northwards and southwards, as well as in places with high altitudes (188). Dengue, which traditionally occurred between latitudes 35° north (N) and 25° south (S), has now been reported as far north as 45°N and as far south as 35-40°S, particularly during warmer seasons (186). Areas previously considered low-risk for dengue, such as Mediterranean Europe (189), continental United States of America (190, 191), and China (192), have in recent years encountered autochthonous dengue cases. Nepal has also reported an increasing number of cases in populations living at altitudes even higher than previously encountered (193).

The combination of climate change and increased urbanization has thus resulted in the geographical expansion of environmental suitability for the vector and subsequent increase in the human population at risk of dengue.

Dengue in travelers

With an increase in the number of travelers to dengue endemic regions, such as Southeast Asia, for both work and leisure, the incidence of travel-associated dengue cases has increased (194), along with more cases of severe and fatal dengue (195, 196). Likewise, dengue-infected travelers from endemic regions to places with established suitable vectors may seed the spread of dengue and consequent outbreaks (192, 197).

Besides increased dengue incidence, the geographic expansion of dengue into new regions poses a second problem. Low awareness of dengue could delay clinical recognition and, hence, appropriate diagnosis and management of dengue patients. Delayed diagnosis could also affect the timeliness of public health response to prevent local DENV transmission and outbreaks. Furthermore, case management may not adhere to guidelines, again due to the lack of familiarity with dengue among healthcare professionals in non-endemic settings (198).

Changing population structure in dengue endemic regions

Even in dengue-endemic regions, changes in the demography of dengue cases can also complicate diagnosis and management. In particular, the incidence of dengue in older adults is increasing.

Different factors have contributed to the changing demography of dengue patients in different places. First, in places with a long history of dengue control, such as Cuba and Singapore, reducing the transmission for decades has paradoxically increased the proportion of adults who remain vulnerable to symptomatic DENV infection. The increased susceptibility to dengue with dengue control can be seen from the series of serological surveys, showing lowered seroprevalence rate over the years in Singapore (199–202), since the inception of its *Aedes* control program in 1970 (10, 12). In Singapore, adult dengue cases now form nearly 90% of all reported cases (8). Furthermore, with an aging population, the proportion of dengue cases in individuals 55 years and older constitutes approximately 20% of all cases (203). Similarly, in Cuba, dengue outbreaks have affected older adults, with disproportionately increased risk of severe disease (204, 205).

Besides dengue control, changes in population structure due to immigration and emigration in dengue endemic regions can alter the demographic structure of the

population that is susceptible to dengue (206). Likewise, lowered birth rates have reduced the force of infection, result in an increasing age of dengue patients in Thailand; the mean age of dengue patients has shifted from 8 years in 1981 to 24 years in 2017 (22).

The collective effects of the expansion of dengue into previously non-endemic places, changes in population demographics, and reduced transmission rate from mosquito control programs in a limited number of places are that dengue in adults and especially older adults has become more prevalent (203). As dengue pathogenesis closely involves the immune response to infection, an aging immune system may add nuances to the pathobiology of dengue in older adults. Aging affects both the innate and adaptive immune systems (207, 208). Changes have been observed in the activation of neutrophils, NK and NK T cells, monocytes and macrophages, dendritic cells, as well as B cells and T cells (209). Furthermore, senescent monocytes may upregulate DC-SIGN expression and entry receptor that increases susceptibility to DENV infection (210). The impact of the aging immune system on dengue pathogenesis remains to be explored in detail.

NEW CASE MANAGEMENT CHALLENGES

Dengue in the older adult

Although the extent to which an aging immune system alters dengue pathogenesis is unclear, managing dengue in older adults presents clear clinical challenges. Current case management approaches have been developed through studies in pediatric dengue cases (142, 211). Children are at greater risk of uncompensated plasma leakage as even under healthy conditions, the rate of plasma leakage is highest in the early years of life (212); superimposition of DENV infection-induced cytokine response that exacerbates leakage thus increases the risk of shock. With increasing age, however, the risk of uncompensated plasma leakage is lower. There are also clinical nuances between plasma leakage in children compared with adults. In a systematic review and meta-analysis, pleural effusion was much more common in children (defined as 17 years old and younger) compared with adults at 43% versus 13% (213). In addition, the occurrence of pleural effusion was associated with severe dengue in children but not adults (213). Other manifestations of plasma leakage, such as ascites, also appear to be more common in children compared with adults (214). However, adult dengue patients may be at greater risk of other complications, including internal bleeding and organ dysfunction (215, 216). Consequently, despite the differences in plasma leakage, older adults remain at risk of severe dengue outcomes, including mortality (217–221).

The initial presenting signs and symptoms of dengue also appear to differ between pediatric and adult patients. Children more commonly present with symptoms such as fever, rash, and pruritus, whereas adults are more likely to present with symptoms such as headache, myalgia, and arthralgia (222–224). Nuances can also be found in the platelet count and serum biochemistry (216, 222–225). Similarly, there are also differences in the rate of vascular leakage and internal bleeding between children and adults with dengue (216, 226). Table 1 provides an overview of these differences.

Besides the differences in clinical features between dengue in adults compared with children, older adults with dengue bring additional challenges. Older adults with dengue may present with highly attenuated symptoms (227). Consequently, the sensitivity of the WHO diagnostic criteria for dengue is lower in older compared with young adults (227). Furthermore, as dengue itself mostly presents as an acute undifferentiated fever and cannot be easily differentiated from other causes of acute febrile illness (228), atypical presentation may further hinder timely diagnosis and management in older adults.

In the absence of a licensed antiviral drug against dengue, treatment for dengue patients remains primarily supportive. In this context, it is noteworthy that, despite the differences in plasma leakage and severe dengue between children and adults (213), the clinical management of older adults remains extrapolated from those developed for the pediatric population. In particular, randomized clinical trials on fluid replacement to

TABLE 1 Differences between pediatric and adult dengue

	Children	Adults	References
Clinical presentation	More common symptoms: – Vomiting – Rash – Pruritus	More common symptoms: – Headache – Myalgia – Arthralgia – Retro-orbital pain – Nausea	(222–224)
Hematology and serum biochemistry	– Less severe thrombocytopenia	– More severe thrombocytopenia – Higher serum alanine transaminase levels	(216, 222–225)
Complications	– Vascular leakage is common – Higher risk of hypovolemic shock	– Vascular leakage is less common – Higher risk of internal bleeding	(216, 226)

support blood pressure during plasma leakage, which is central to dengue management, have thus far only been conducted in pediatric dengue patients (229–233).

Systematic review and meta-analyses have consistently found that co-morbidities that affect the vascular health of patients, such as obesity (234), diabetes, renal disease, hypertension, and cardiovascular disease, are associated with severe dengue and death (235). Besides worsening risks of severe dengue, pre-existing cardiac or renal conditions further complicate the management of the fluid balance critical to optimal outcomes of dengue patients with severe plasma leakage (236). Perturbations of baseline physiology and medications used in the treatment of cardiac and or renal conditions, such as beta-blockers or diuretics, could also make it difficult to monitor for complications and timely medical intervention. For example, beta blocker use may block tachycardic response in shock and mask intravascular hypovolemia. Patients with chronic renal failure or heart failure are likely to have limited fluid tolerance, and standard fluid replacement regimens may result in fluid overload. Urine output measurement, which is recommended as part of standard dengue monitoring, may be inaccurate as an indicator of intravascular volume status in this group of patients. Unfortunately, no specific guidance on fluid replacement currently exists for dengue patients with underlying cardiac or renal failure.

Compared with pediatric dengue patients, adult patients often develop more significant thrombocytopenia (216). Platelet counts of <20,000 /mm^3 are not uncommon during the critical phase of acute dengue, although bleeding manifestations are generally mild and self-limiting in most patients (237–239). A small minority, however, do go on to develop clinically significant hemorrhage, often in the context of prolonged shock, coagulopathy, or pre-existing co-morbidities, including chronic liver or kidney disease (240, 241). Current evidence does not support routine use of prophylactic platelet transfusion in patients with dengue, with several studies demonstrating that platelet transfusion did not prevent bleeding, hasten platelet recovery, or improve clinical outcomes (239, 242). Even in adult dengue patients whose platelet counts were <20,000 /mm^3, prophylactic platelet transfusion not only showed no benefit but also was associated with a higher incidence of transfusion-related adverse events (239). Thus, platelet transfusion should be reserved for patients with active bleeding or those requiring invasive procedures where bleeding risk is deemed significant.

Dengue-induced thrombocytopenia may also present significant challenges in the management of patients requiring mandatory anticoagulation, such as those with prosthetic heart valves. In these individuals, the risk of bleeding must be balanced against the potentially life-threatening consequences of thromboembolic events following interruption of anticoagulation therapy. Currently, there are no specific evidence-based guidelines for the management of anticoagulation or antiplatelet

therapy in the context of dengue-induced thrombocytopenia. As such, clinical decisions are largely individualized, based on an assessment of bleeding risk versus thrombotic risk, which depends on the underlying indication for anticoagulation, co-morbid conditions, and duration of therapy interruption (243–245). In such cases, a multidisciplinary approach involving infectious disease physicians, hematologists, and cardiologists may be warranted to guide optimal management. Clinical challenges in managing adults and especially older adults with dengue are summarized in Fig. 7.

Although co-morbidities complicate dengue, the corollary is also true. Dengue complicates pre-existing chronic diseases. Several studies have found increased risk of cardiovascular events, including heart failure and stroke, in adults after recovering from dengue (246, 247). Dengue could also worsen the risk of autoimmune disease (248), dementia (249–251), and other psychiatric disorders or lead to chronic sequelae (252, 253).

Dengue in pregnancy

Adults susceptible to DENV infection would include pregnant women. Maternal dengue patients may show attenuated symptoms and signs, making diagnosis challenging (254). A systematic review found that maternal dengue was associated with increased risk of preterm delivery (255), with a separate study showing that the odds of preterm delivery and low birth weight increased with severity of maternal dengue (256). Besides posing risks to the newborn, dengue during pregnancy may also adversely affect maternal health, with an increased likelihood of obstetric complications (257), frequent hospitalizations (258), and mortality from severe dengue (259).

The published literature on dengue during pregnancy, however, is littered with small-scale case-control studies and case reports, which may be confounded by biases; there have been few prospective studies (255). Moreover, the available evidence thus far has not consistently demonstrated an increased risk of severe

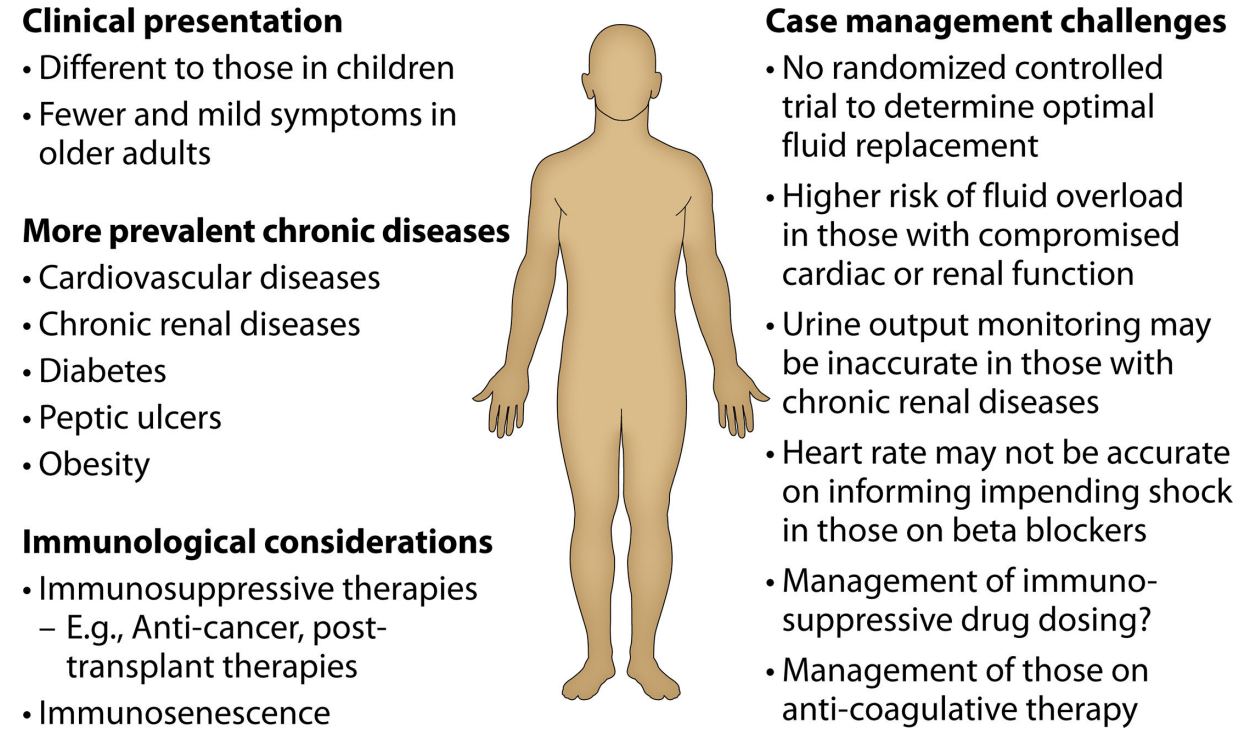

Clinical presentation
• Different to those in children
• Fewer and mild symptoms in older adults
More prevalent chronic diseases
• Cardiovascular diseases
• Chronic renal diseases
• Diabetes
• Peptic ulcers
• Obesity
Immunological considerations
• Immunosuppressive therapies
– E.g., Anti-cancer, post-transplant therapies
• Immunosenescence
Case management challenges
• No randomized controlled trial to determine optimal fluid replacement
• Higher risk of fluid overload in those with compromised cardiac or renal function
• Urine output monitoring may be inaccurate in those with chronic renal diseases
• Heart rate may not be accurate on informing impending shock in those on beta blockers
• Management of immuno-suppressive drug dosing?
• Management of those on anti-coagulative therapy

FIG 7 Challenges in managing older adults with dengue. Older adults present with different symptoms and even attenuated symptoms compared to children with dengue. Older adults are also more likely to have co-morbidities and be taking medication that places them at greater risk of severe dengue and complicate case management.

dengue in pregnant women, likely reflecting the multifactorial nature of severe dengue pathogenesis (260).

Dengue in immunosuppressed

With age, the prevalence of dengue among immunosuppressed individuals has also become more common. In people living with human immunodeficiency virus (HIV) infection (PLHIV), there was initial concern that the pre-existing and persistent pro-inflammatory state of PLHIV would worsen the risk of severe dengue (261). However, clinical studies (262) and a meta-analysis (263) have not found worse outcomes but instead reduced risk of severe dengue. Like PLHIV, an immunocompromised state from malnutrition also does not appear to increase the risk of severe dengue (263). However, it remains unclear if there is an increased risk of poor outcomes, which may be due to the breadth and multifaceted nature of immunodeficiency within malnutrition.

In contrast to PLHIV and those with malnutrition, the opposite has been observed in solid organ transplant (SOT) recipients (264). Dengue in SOT recipients can be prolonged, with prolonged viremia (265); DENV infection was detectable in the blood and urine of a SOT recipient even at 4 and 9 months, respectively, after symptom onset (266). Besides *Aedes*-transmitted infection, donor-derived DENV infections, from both cadaveric donors and living donors, have also been reported (254, 267–269). Such cases also show prolonged infection and have poor clinical outcomes (264). Clinicians managing such patients often face the challenge of balancing immunosuppressive therapy to preserve graft function while enabling sufficient immune response against DENV to facilitate infection resolution (266). Indeed, a review from Singapore of 31 renal transplant recipients with dengue found an increased risk of graft dysfunction, although in most cases, this was mild and transient (270).

Unlike SOT, the clinical outcome of dengue in hematopoietic stem cell transplant recipients remains to be clearly defined (271).

DENGUE THERAPEUTICS

Antiviral drug discovery and development

There is no antiviral treatment for dengue patients. Discovery efforts for a treatment for dengue can be broadly divided into two categories: inhibition of DENV infection and inhibition of host response to infection that contributes to pathogenesis. A detailed list of antiviral drug candidates that have either been tested in early phase clinical trials or in pre-clinical development stages or have been reviewed elsewhere (272). It is noteworthy that thus far, all but one compound that has been tested in clinical trials has failed to show efficacy in reducing viremia in dengue patients. Thus, rather than discussing compounds that have been tried and failed, this section will instead focus on the challenges faced by antiviral discovery and development for the treatment of dengue.

Inhibiting DENV infection can either be through discovering compounds that either directly bind viral proteins, also referred to as direct-acting antiviral (DAA) compound, or inhibit host factors required for DENV infection (273, 274). Either approach would require the inhibitor to act on all four DENVs with equal potency or on a host factor that is shared by all four DENVs. Furthermore, as severe dengue occurs only in a small fraction of all dengue patients, the first hurdle any drug candidate will have to overcome, besides safety, is efficacy in reducing viremia (275, 276). The assumption that a drug that can reduce viremia would concomitantly reduce dengue severity is reasonable, as high viremia at diagnosis has been shown to correlate with severe dengue (140). However, treatment would have to be started early, as only viremia levels in the first 48 h from illness onset correlated with severe dengue outcome (141). Nonetheless, an antiviral therapy that shortens viremia can be effective as those with shorter duration viremia have reduced risk of severe dengue, compared to those with long duration of viremia (141). The key is early dosing (Fig. 8).

Direct-acting antiviral drug discovery

Any treatment to prevent severe dengue early, at a time when the illness is undifferentiated, will require a drug that is safe, easy to administer, and readily available in ambulatory clinics. Such a drug would preferably be orally delivered, although intravenous formulation may still be necessary for dengue patients with vomiting. Besides suitable compounds and formulations, considerations will also be needed for pre-clinical studies. Most pre-clinical evaluation of antiviral compounds in animal models initiated dosing at the time of infection or soon after infection. Such dosing approaches ignore the fact that in dengue patients, viremia would be near peak or even on a waning trajectory at the time of diagnosis. Premature dosing in animal models would thus over-estimate the efficacy of any compound in reducing viremia in dengue patients.

Clinical trials on dengue antiviral candidates have also endeavored to enroll dengue patients within the first 48 or 72 h from illness onset (153, 277). However, enrolling dengue patients within this small window of opportunity is challenging, given that early symptoms are mostly undifferentiated. Many have thus used point-of-care tests for DENV sNS1 as a screening tool to differentiate dengue patients from those with other febrile illnesses. Although the test kit is highly specific, it lacks sensitivity in diagnosing dengue in patients with secondary DENV infections; the sensitivity drops from over 99% in those with primary dengue to approximately 60% in those with secondary dengue (278). Paradoxically, those with secondary dengue are at greater risk of severe disease than those with primary dengue, and are hence more in need of treatment. Although nucleic acid amplification tests (NAATs), such as DENV reverse transcriptase polymerase chain reaction (RT-PCR), enable early diagnosis with high sensitivity and specificity, they are currently not widely available, especially in resource-limited settings (279).

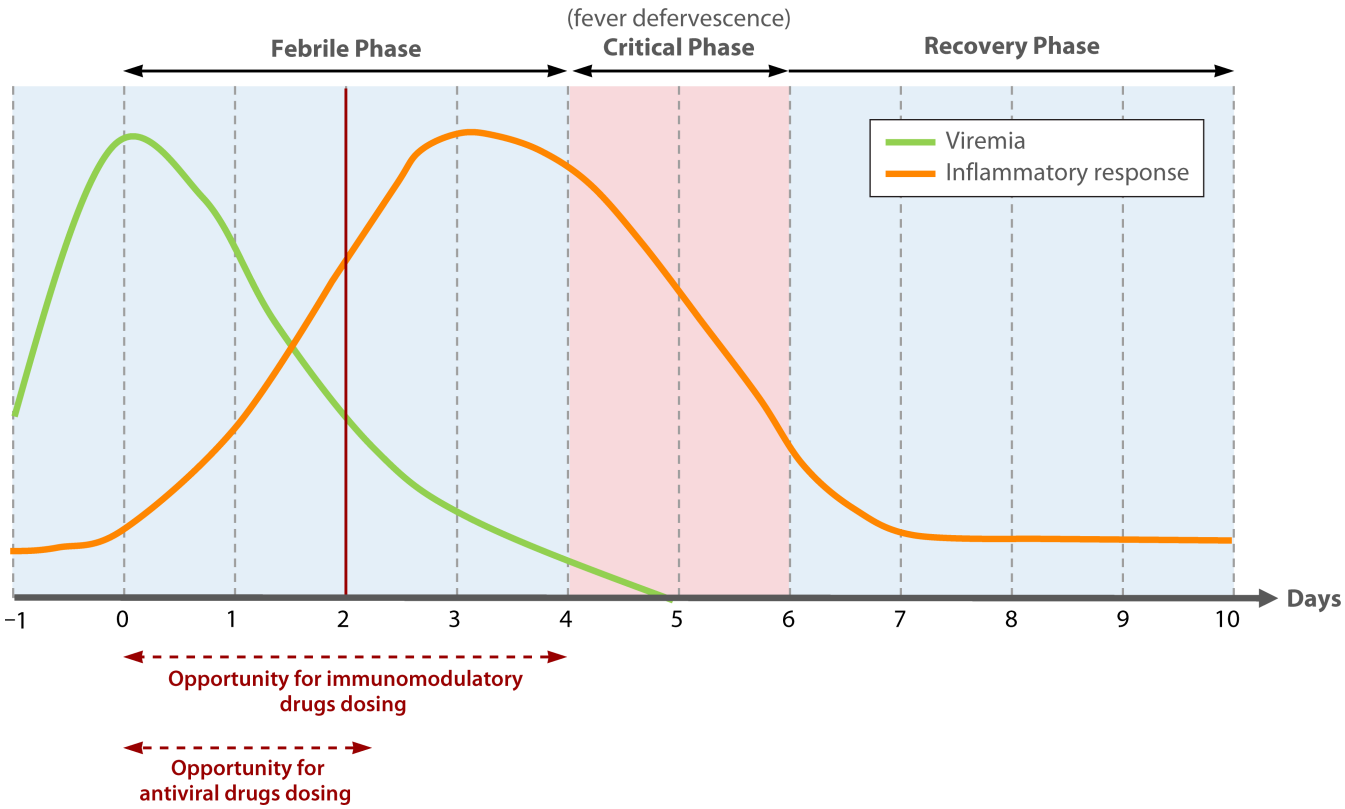


FIG 8 Kinetics of viremia and pro-inflammatory response shape therapeutic interventions. DENV viremia (green line) begins before and peaks around the time of illness onset. Viremia levels decline rapidly after illness onset. Theoretically, DAA has to be administered as early as possible during the viremic phase. Pro-inflammatory responses (orange line) to the DENV burden occur slightly later but peak before fever defervescence, where plasma leakage is maximal. Inhibitors of pro-inflammatory response must thus be given before severe dengue develops.

Despite these challenges, attempt has been made to develop several DAA drugs to treat dengue patients. Attractive targets for DAA are obviously the active sites of key viral enzymes involved in replication, such as the protease and helicase in the NS2B/NS3 protein complex, and the methyltransferase and polymerase in NS5 protein (280). Among these, several polymerase inhibitors have undergone clinical development. NITD-008, an adenosine analog, showed promising antiviral activity in both *in vitro* and *in vivo* studies. However, its development was halted due to safety concerns identified during pre-clinical toxicology studies, including hematological and hepatic toxicity (281). Another polymerase inhibitor, balapiravir, was tested in a proof-of-concept clinical trial against dengue but showed no efficacy (282); subsequent *in vitro* investigations suggest that balapiravir, as a pro-drug, failed to be converted to the active molecule in DENV-infected primary monocytes (283). A phase 2 clinical trial of AT-725, also an NS5 inhibitor developed by Atea Pharmaceuticals, has been terminated due to challenges in enrolling sufficient numbers of dengue patients early in the course of illness (284).

Although targeting the active sites of viral enzymes is the most intuitive approach for antiviral drug discovery, the two small molecules that have shown the greatest promise to date are instead directed against the NS4B protein. NS4B has no enzymatic function but is an integral part of the replication complex (42). Two small molecules, JNJ-1802 (developed by Johnson and Johnson) and NITD688 (developed by Novartis), have advanced to phase 2/3 and phase 2 clinical trials, respectively. Both compounds demonstrated inhibition of DENV replication at low nanomolar concentrations (285–287). Unfortunately, in a recent development, Johnson and Johnson have terminated clinical development of JNJ-1802 due to strategic re-prioritization (288).

Besides small molecules, attempt is also being made to develop a monoclonal antibody (mAb) that neutralizes all four DENVs as a therapeutic against dengue (289). This humanized mAb, under clinical development by Serum Institute of India, has shown potentially useful efficacy in pre-clinical studies (290) and is currently being tested in clinical trials (291). However, it remains to be determined whether a therapeutic mAb that is delivered by intravenous infusion can be both affordable and accessible to dengue patients early in the febrile phase of illness and thus enable management in ambulatory settings (292).

Host-directed therapies

Systemic inflammatory response to infection is an important contributor to endothelial dysfunction and plasma leakage. Indeed, plasma concentration of pro-inflammatory cytokines have been found to track with the magnitude of viremia and persist in patients with severe dengue even after viremia resolves during the defervescence period, when shock mostly occurs (140, 141, 293). The window of opportunity for immunomodulatory treatment may thus be wider than that for DAA (Fig. 8).

Several cytokines have been associated with plasma leakage and severe dengue (294–297), among which are IL-1, IL-6, and TNF, which are potential targets as there are licensed drugs that specifically target these cytokines. Small molecules that act on pathways that lead to expression of these cytokines or effect the functions of these cytokines could also be explored as therapeutics for severe dengue.

Interleukin-1 (IL-1) has been implicated in dengue pathogenesis (298–300). A higher peak plasma IL-1 receptor antagonist (IL-1RA) concentration (surrogate marker for IL-1 activity) in the early phase of dengue was recently found to be associated with more severe clinical outcomes (140). The bio-engineered IL-1RA Anakinra is a licensed drug that blocks IL-1 signaling. It is used to treat auto-inflammatory conditions such as rheumatoid arthritis and has also shown efficacy in reducing the risk of severe COVID-19 (301–304). A placebo-controlled clinical trial is currently underway at the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam, to evaluate the efficacy of Anakinra in preventing severe dengue (NCT05611710).

Besides, IL-1, the pro-inflammatory cytokine IL-6 was also positively associated with vascular leakage (296, 297, 305–307). IL-6 signaling can be inhibited through the use of

an inhibitor of IL-6 receptor Tocilizumab (308), a licensed treatment of several autoimmune diseases (309) and cytokine release syndrome (CRS) (310). Tocilizumab has also shown benefit as treatment to reduce the risk of severe COVID-19 in patients with high plasma IL-6 levels at enrollment (311, 312).

Finally, another potential target is tumor necrosis factor (TNF). TNF is a pro-inflammatory cytokine that is also involved in increasing adhesion molecule release and endothelial permeability (313). Patients with severe dengue appear to have higher TNF levels (314). In a prospective study carried out in the Philippines, elevated soluble TNF receptor-1 appeared predictive of hospitalization among outpatients with DENV infection (315). Moreover, anti-TNF immunoglobulin treatment has reduced severity of dengue in mouse models (316–318). Drugs, such as infliximab, adalimumab, and golimumab (319), could thus modulate inflammation and prevent endothelial activation for improved outcomes in dengue patients.

To date, the only completed immunomodulatory treatment trial has been high-dose prednisolone (320). Corticosteroids dampen pro-inflammatory responses but could also attenuate antiviral responses (321). Furthermore, the phase 2 clinical trial on prednisolone was conducted in an unselected patient population, most of whom had a high likelihood of uncomplicated dengue (320, 322). Indeed, important lessons can be drawn from previous failed sepsis trials, and more recently from COVID-19 trials, that highlight the need to identify and target appropriate patient sub-populations to demonstrate clinical benefit while minimizing potential harm from immune modulation (323, 324). Triaging of patients based on risk of severe dengue at presentation or hospitalization will be needed for both clinical trials as well as successful use of immunomodulators (6) (Fig. 8). Although enrolling mild dengue patients would dilute the number of events required for measurable outcome, recruiting patients in the critical phase of illness or those who have already manifested severe dengue could miss the limited time window for intervention (Fig. 8). Mediators that drive early pro-inflammatory states could also differ from those at the critical phase driving a dysregulated immune response (325–327).

In conclusion, there remains an urgent need for antiviral and anti-inflammatory treatments for dengue patients. At the same time, there is a need for better point-of-care diagnostic and prognostic tests to facilitate both clinical trials of therapeutic drugs and eventual triaging of dengue patients at risk of severe dengue for therapy.

DENGUE VACCINES AND IMMUNITY

State of play of dengue vaccines

Dengue vaccine development has made significant progress over the past two decades, with several candidates reaching advanced stages of clinical development and even licensure. However, the unique immunopathogenesis of dengue has posed significant challenges for the design of safe and broadly efficacious dengue vaccines (13). Indeed, none of the three tetravalent live-attenuated dengue vaccines licensed to date or that have completed Phase 3 clinical trials have demonstrated complete protection against symptomatic infection from all four DENVs, particularly in those without prior DENV infection, or seronegative individuals (328–335) (Fig. 9). While achieving such comprehensive protection remains an aspirational goal, there is nonetheless cause for optimism; at least two of these vaccines have demonstrated good overall efficacy and, crucially, have not shown an increased risk of severe dengue in seronegative vaccinees (334, 335). This now makes dengue a vaccine-preventable disease, with the potential to bring significant public health benefits, particularly in dengue-endemic settings.

The first dengue vaccine to be licensed was CYD-TDV. Developed by Sanofi-Pasteur, CYD-TDV is a recombinant, live-attenuated vaccine based on an attenuated yellow fever 17D (YF17D) vaccine virus backbone expressing the prM and E proteins of the four DENVs (339). Although CYD-TDV demonstrated good efficacy in preventing virologically confirmed dengue (VCD), that is, symptomatic RT-PCR positive dengue, it showed limited to no efficacy against DENV-2 in seronegative children (328, 329). Although initially

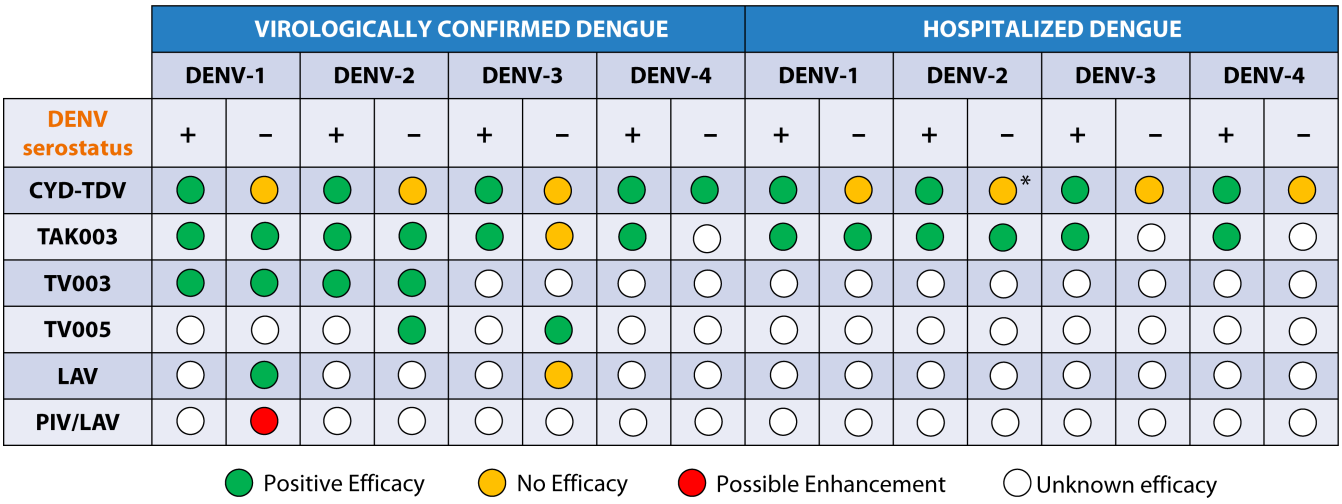


FIG 9 Efficacy of tetravalent dengue vaccine candidates. Vaccines that have completed phase 3 clinical trials with long-term follow-up (CYD-TDV, TAK003, TV003) or have been tested in a human challenge study (TV005, LAV, PIV/LAV). Efficacy is categorized by dengue serostatus of vaccinees, cross (+) symbol indicates sero-positivity and dash (-) symbol indicates sero-negativity. Efficacy against each of the 4 DENVs is categorized using colored circles (green: positive efficacy; yellow: no efficacy; red: possible enhancement; white: unknown efficacy) based on the longest study follow-up period for which data is publicly available. For CYD-TDV, efficacy shown is for participants aged 9-16 years. *Possible enhancement in children aged 2-8 years. Sources of information: CYD-TDV (330); TAK-003 (334); TV003 (335); TV005 (336); Live-attenuated vaccine (LAV) (337); and purified inactivated vaccine (PIV) followed by LAV (338).

licensed for use regardless of dengue serostatus, subsequent post-hoc analyses from the Phase 3 clinical trial revealed an increased risk of dengue-related hospitalization after the second year of follow-up among seronegative vaccinees without prior dengue exposure (330). This led to revised recommendations restricting its use only to individuals with confirmed past dengue exposure. Although to date, there has been no real-world evidence conclusively demonstrating an increased risk of severe dengue in CYD-TDV vaccinees, the perception of harm, particularly in children, has contributed to vaccine hesitancy not only toward CYD-TDV but also toward newer dengue vaccine candidates (340, 341). Additionally, as a result of poor global uptake and constrained application, Sanofi has announced plans to discontinue manufacture of CYD-TDV by the third-quarter of 2026.

The second vaccine to achieve licensure was TAK003. TAK003, developed by Takeda Pharmaceuticals, is a live-attenuated vaccine based on an attenuated DENV-2 (PDK53) backbone engineered to express the prM and E proteins of all four DENVs (77). This two-dose vaccine has now been licensed in several countries. A phase 3 trial involving over 20,000 participants aged 4–16 years across eight countries reported an overall efficacy of 80.2% against VCD (331), and 90.4% efficacy against hospitalized dengue (332), although efficacy varied by the type of DENV and baseline serostatus. Efficacy was highest against DENV-2 followed by DENV-1 in seropositive individuals, but there was no efficacy against DENV-3 in seronegative individuals. Efficacy against DENV-4 in seronegative individuals remains unknown, as there were too few cases during the clinical trial. Reassuringly, however, long-term 4.5-year follow-up of study participants has demonstrated sustained efficacy against hospitalized dengue regardless of serostatus, with no evidence of an increased risk of severe dengue in seronegative vaccinees (334). As such, this vaccine has been licensed for use even in individuals without serological evidence of past dengue infection.

The third vaccine now awaiting licensure is Butantan-DV. This single-dose vaccine, developed by Instituto Butantan, is a live-attenuated tetravalent formulation derived from the U.S. National Institutes of Health (NIH) candidate TV003. TV003 is constructed by deleting 30 nucleotides in the 3′-UTR of DENV-4 and DENV-1 (rDEN4Δ30 and rDEN1Δ30), and 30 and 31 nucleotides from DENV-3 (rDEN4Δ30/31) (342). The DENV-2

component was constructed by swapping the DENV-2 prM and E genes into the rDEN4Δ30 backbone. The phase 3 clinical trial conducted in Brazil enrolled over 16,000 participants aged 2–59 years (333). Overall efficacy was 79.6% against VCD in the first 2 years of follow-up, with 89.2% and 73.6% efficacy in seropositive and seronegative individuals, respectively. When stratified by DENV type, vaccine efficacy against DENV-1 was 96.8% in seropositive individuals and 85.6% in seronegative individuals, whereas efficacy against DENV-2 were 83.7% and 57.9%, respectively. Efficacy against hospitalized dengue was not reported. In addition, there were no cases of DENV-3 and DENV-4 in participants during the clinical trial to determine efficacy against either of these two DENVs. Extended follow-up of trial participants for up to 2–5 years (median: 3.7 years) has demonstrated reasonably sustained efficacy against both DENV-1 and DENV-2, even in seronegative individuals (335).

Although Instituto Butantan is the most advanced in the clinical development of TV003, this vaccine formulation has also been licensed by the NIH to other entities for further development. Notably, Merck & Co.'s (MSD outside the USA) investigational candidate V181, which includes two formulations, TV003 and TV005 (the latter containing a higher dose of the DENV-2 component), has demonstrated promising immunogenicity and safety profiles in phase 1 studies (343), supporting its continued clinical development. The efficacy of TV005 has also been tested using a human challenge model and has demonstrated complete protection against partially attenuated strains of both DENV-2 and DENV-3 (336).

Correlates of protection against dengue

As defined by Plotkin, a correlate of protection (CoP) is "an immune function that correlates with and may be responsible for vaccine-induced efficacy," encompassing both immune responses that are directly biologically responsible for protection and those that may merely be a statistical association, sometimes also referred to as a "surrogate of protection" (344, 345). A CoP is useful as an immunological endpoint to select vaccine candidates for further clinical development. Identifying reliable CoPs against DENV infection, however, remains a major challenge. Despite decades of research, no single correlate (or surrogate) has been universally validated for dengue, reflecting the complexity of dengue immunopathogenesis and immunity.

Neutralizing antibodies

Neutralizing antibodies (nAbs) have long been considered the principal immune CoP against orthoflaviviruses, including DENVs. Early studies in non-human primates by Halstead et al. demonstrated that the presence of nAbs correlated with protection against viremia following experimental DENV challenge (346, 347). Similarly, longitudinal cohort studies in both children and adults have shown that higher nAb titers are associated with a reduced risk of symptomatic DENV infection and severe disease (348–350). However, this association is not absolute and may be modulated by factors such as an individual's history of prior DENV infections and the infecting DENV type. A recent study demonstrated this complexity (351) - Analysis of longitudinal serological samples from a Nicaraguan pediatric cohort showed that high pre-infection nAb titers conferred protection only in individuals with a single prior DENV infection, but not in those with two or more previous infections (351). Moreover, protection appeared to be DENV type-specific, with high pre-infection nAb titers associated with reduced risk of symptomatic infection from DENV-1 and DENV-2, but not DENV-3 (351).

To date, no single nAb threshold has been established as a CoP for dengue vaccination. Defining such a threshold is challenging, as even individuals with very high nAb titers may still develop disease (352). Protection against dengue is further complicated by the uncertainty on the range of antibody titers where the risk of ADE is increased (101, 129, 353). In addition, protective nAb thresholds likely differ by DENV type. Observational studies and post-hoc analysis of serological data from CYD-TDV phase 3 trials suggest that DENV-2 requires higher nAb titers for protection compared with DENV-1 and DENV-3

(349, 352). This may reflect DENV-2's higher intrinsic risk for ADE and severe clinical outcomes. Indeed, multiple observational studies have shown that secondary DENV-2 infection is more frequently associated with symptomatic disease and severe outcomes such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (354–356). Consistently, in a cohort of over 5,000 DENV-infected patients, it was demonstrated that within viremia ranges expected during the early febrile phase, the risk of plasma leakage and severe disease was greatest for secondary DENV-2 infections compared with other DENVs (13, 140).

Beyond nAb quantity, nAb quality may also be an important factor influencing protection against dengue, specifically in the context of vaccination. It has been suggested that the induction of type-specific nAbs may be critical for achieving durable, type-specific immunity from vaccination. The rationale for this line of thinking stems from two observations: discovery of potent type-specific nAbs in those who have recovered from a primary DENV infection and that those who have experienced one DENV infection would mostly be protected against homologous re-infection (104, 204, 357). However, the role of type-specific versus cross-reactive antibodies remains complex; cross-reactive monoclonal antibodies that bind all four DENVs have shown potent infection neutralization activities (358, 359). Assessment of nAb functional quality, however, is challenging. Conventional plaque reduction neutralization titer (PRNT) assays may be limited in distinguishing between high- and low-quality nAbs (344, 360), although recent advances, including assays that use mature DENV particles and cellular substrates such as Vero cells engineered to express DC-SIGN, may provide improved resolution of functional nAb responses (351, 361).

The hypothesis that vaccines must induce type-specific nAbs against all four DENVs to achieve balanced type-specific efficacy remains somewhat debatable. Indeed, evidence from phase III clinical trials of CYD-TDV, TAK-003, and TV003 (Butantan-DV) remains mixed. For CYD-TDV and TAK-003, the generation of type-specific antibodies corresponded with higher type-specific vaccine efficacy (362–365); CYD-TDV primarily elicited type-specific responses against DENV-4 (362), whereas TAK-003 predominantly induced responses against DENV-2 (364, 365), mirroring the higher efficacy observed against DENV-4 and DENV-2, respectively. However, findings from the TV003 trial somewhat challenge this association. TV003 achieved relatively balanced type-specific nAb responses across all four DENVs, thought to be due to the ability of all four of its vaccine strains to cause viremic infection (366–369). Nevertheless, a notable disparity in efficacy was observed among seronegative vaccinees, with protection against DENV-1 reaching 85.5% compared with only 57.9% against DENV-2 (333). This gap widened over longer-term follow-up, with vaccine efficacy against DENV-1 and DENV-2 in seronegative individuals declining to 73.2% and 43.6%, respectively (335). These observations suggest that although type-specific nAbs may contribute to protection, the minimum titer for protection against symptomatic infection may differ across the four DENVs.

Fc-mediated functions

The role of Fc-mediated effector functions in dengue disease enhancement has been discussed in detail in earlier sections of this review. Limited emerging evidence now also suggests a protective role for Fc-mediated mechanisms, specifically complement-mediated immunity. In a recent study, higher titers of pre-infection total IgG and IgG4 antibodies against DENV E and NS1 were found in asymptomatic compared with symptomatic children infected with DENV-3 (370). This protection appeared to be mediated by antibody-dependent complement deposition, resulting in lysis of both free virions and DENV-infected cells. This finding is consistent with prior *in vitro* and animal studies demonstrating complement-associated protection in orthoflaviviral infections, including protection against ADE (371–373). Interestingly, in the same cohort, complement-mediated lysis of Zika virus (ZIKV) virions, using plasma from DENV-immune but ZIKV-naïve children, correlated with protection from subsequent severe dengue outcomes including DHF and DSS (374). Although suggested as a possible CoP for

dengue, the mechanistic relevance of this observation to DENV-specific viral control remains to be validated.

In addition to complement-mediated mechanisms, other antibody-dependent pathways may also contribute to protection. DENV-reactive IgA has been shown to antagonize IgG-mediated ADE, offering a potential mechanism for modulating disease severity (121, 122). Furthermore, Fc-mediated cellular responses may also play a role. NK cells, which mediate antibody-dependent cellular cytotoxicity (ADCC), have been implicated in protection during acute infection (138, 168). In a Vietnamese cohort of hospitalized dengue patients, those with no or only mild plasma leakage exhibited higher frequencies of activated NK cells expressing perforin and granzyme compared to individuals with more severe disease and evidence of systemic hyperinflammation, although functional assays to directly assess NK cell cytotoxicity could not be performed due to limited blood volumes (168). Conversely, dysfunctional NK cells with increased co-inhibitory receptor expression and decreased cytotoxic potential have been found in patients with severe dengue compared with those with non-severe dengue (375).

Overall, there is a growing body of evidence to suggest that protective Fc-effector functions during DENV infection may extend to include both complement activation and cytolytic cellular responses, although further studies are warranted in this area.

Cellular immunity

Emerging evidence, particularly from SARS-CoV-2 research, has highlighted the critical role of T cells in mediating protection against acute viral diseases and supports their inclusion as a CoP in viral disease and vaccination. Indeed, T cells, rather than nAbs, represent the key CoP against breakthrough SARS-CoV-2 infection, providing a foundational layer of immunity until hybrid immunity is established (376). Moreover, early memory T cell expansion correlates with rapid viral clearance and reduced disease severity (377–379).

In orthoflaviviral infection, the importance of T cell immunity is increasingly recognized. Indeed, the excellent and durable efficacy of the YF17D vaccine has been at least partially attributed to its ability to elicit broad and polyfunctional antigen-specific T cell responses (380–383). Critically, experimental evidence from both animal models (384–389) and now more recently in human experimental infection (390) has demonstrated the ability of T cells to mediate viral control independent of nAbs. Specifically, T cells directed against the C protein were associated with a level of viral control that was to the extent of sterilizing immunity (390).

The role of T cells in dengue has been somewhat contentious. Earlier hypotheses posited that antigen-specific T cells induced during a primary DENV infection could drive the expansion of low-affinity, cross-reactive memory T cells during secondary heterologous infection. This was believed to result in increased immunopathology due to inability to clear virus, and secretion of pro-inflammatory cytokines—a phenomenon referred to as "original antigenic sin" (164, 165). However, accumulating evidence now supports a protective role for T cells in dengue. In murine models, cross-reactive T cell responses elicited by primary DENV infection have been shown to contribute to viral control and protection upon secondary heterologous challenge (391, 392). Notably, adoptive transfer of DENV-primed CD8+ T cells reduced viral loads following challenge, even in the absence of nAbs (393). In humans, robust and polyfunctional T cell responses have been associated with reduced risk of severe dengue (394–399). For example, compared with symptomatic DENV patients, asymptomatic but viremic individuals exhibit higher frequencies of activated T cells during acute infection (398), whereas in a separate cohort, unbiased immune profiling revealed that patients with mild dengue had higher frequencies of activated, terminally differentiated T cells compared with hospitalized patients (166). Indeed, the observation that post-secondary DENV infections are often mild or even subclinical may, at least partially, be due to T cells. In a longitudinal pediatric cohort in Nicaragua, children with only one prior DENV infection displayed heterogenous DENV-specific T cell responses, whereas those with two or more

prior infections demonstrated higher frequencies of DENV-specific T cells, which were associated with subclinical or inapparent outcomes upon subsequent infection (399). These findings suggest that T cell responses contribute to protective immunity and may modulate disease severity in dengue.

Beyond absolute T cell counts, the phenotype and functionality of memory T cells activated during acute DENV infection also appear to influence disease outcomes. Activation of CD8+ T cells with an effector memory (T_{EM}) phenotype has been associated with reduced dengue severity (400, 401). In some individuals, DENV-specific CD4+ T effector memory cells re-expressing CD45RA (TEMRA) can acquire cytolytic functions during acute infection (397). These DENV-specific TEMRA cells produce high levels of IFN-γ and granzyme and have been associated with protection against severe disease in secondary DENV infection (402–404). CD4+ T cells also play a complex role in modulating humoral responses during DENV infection. Acute infection is characterized by a massive expansion of plasmablasts, which has been associated with severe outcomes (166, 398, 405). Although this expansion is typically attributed to a T follicular helper (T_{fh}) cell-driven response (406, 407), recent work has identified a distinct population of extrafollicular PD-1^+ IL-21–secreting CD4+ T cells in patients with severe dengue (408). Ex vivo, these cells can drive B cell differentiation into plasmablasts, highlighting non-canonical pathways of B cell help that may contribute to immunopathology. Taken together, these findings support a multi-faceted role for CD4+ T cells in shaping the clinical outcome of DENV infection.

In parallel, the contribution of regulatory T cells (T_{reg}) to dengue pathogenesis is increasingly recognized but remains incompletely understood. Although one study reported that higher frequencies of T_{regs} and an elevated T_{reg} to effector T cell ratio were associated with milder disease (409), others have not observed a consistent correlation with viremia or clinical severity (410). More recent data from a pediatric cohort in Cambodia observed phenotypic and functional alterations of T_{regs} during acute DENV infection compared with non-infected controls (411). Notably, T_{regs} from patients with severe disease exhibited a Th1-like profile and showed diminished suppressive capacity but secreted higher levels of IL-10 upon *in vitro* stimulation. These findings suggest that dysregulated or functionally impaired T_{reg} responses may exacerbate immunopathology, further underpinning the importance of T cell quality across effector, helper, and regulatory subsets in determining dengue disease outcomes.

In the context of dengue vaccination, DENV-specific T cells likely play an important role in preventing severe disease when nAb titers are insufficient to prevent breakthrough infection. In a recent DENV-1 human challenge study in individuals who had received heterologous prime-boost dengue vaccination with a tetravalent purified inactivated vaccine (PIV) followed by live-attenuated vaccine (LAV), the only vaccinee who remained aviremic post-DENV-1 challenge had the second highest total DENV-specific T cell response at the point of challenge infection among all the vaccinees (338, 412). Although the vaccinee with the highest T cell response developed breakthrough infection, they were the only individual to maintain normal full blood count and liver enzyme levels despite viremic infection. Notably, these two individuals had the third and second lowest pre-challenge DENV-1 nAb titers, respectively.

Indeed, the differences observed in the outcome of breakthrough infection in those who have been vaccinated with CYD-TDV, compared with those who received TAK-003 or TV003, may be due to T cells. Unlike nAb epitopes, which are found on the prM and E structural proteins, DENV-specific T cell epitopes, particularly those that are recognized by CD8+ T cells, are primarily found on the C and NS proteins (394). CYD-TDV was constructed using a YF17D backbone and hence does not contain C and NS proteins of DENV. As such, this vaccine elicits mostly YFV-specific, rather than DENV-specific T cells (413). In contrast, both TAK-003 and TV003 use a DENV genomic backbone and thus induce robust and sustained DENV-specific T cell responses that mirror those generated by natural secondary DENV infection (369, 414–419). DENV-specific CD4+ and CD8+ T cells generated by TV003 vaccination appear to initially be of a T_{EM} phenotype followed

by an increase in the frequency of TEMRA cells over time (418); such a phenotype has been associated with protective immunity against DENV (397). It is thus likely that robust vaccine-induced DENV-specific T cell responses could potentially mitigate the risk of severe disease in breakthrough infection in seronegative vaccinees. Indeed, TAK-003 has demonstrated close to 80% sustained efficacy against hospitalized dengue (a surrogate outcome for severe dengue) up to 4.5 years post-vaccination, even in seronegative vaccinees (334). Importantly, unlike CYD-TDV, long-term follow-up of both TAK-003 and Butantan-DV (TV003) phase III clinical trial participants has not demonstrated an increased risk of severe disease (334, 335).

Guidance on vaccine development from dengue immunity

A key lesson emerging from our current understanding of dengue immunity and from the outcomes of phase III clinical trials of CYD-TDV, TAK-003, and Butantan-DV is the need for dengue vaccines to elicit a broad and multi-layered adaptive immune response. Protection against dengue, particularly severe disease, appears to require more than high levels of nAbs. Instead, effective and durable immunity likely depends on a coordinated interplay between humoral and cellular immune components (Fig. 10). As such, the development and evaluation of dengue vaccines must take into account both arms of the adaptive immune response.

Given the immunological complexities of dengue, it is unlikely that any vaccine can achieve complete protection against symptomatic infection from all four DENVs, particularly in seronegative individuals, in the near future. A more attainable goal may be to develop vaccines that can reliably protect against severe disease, even in the context of breakthrough infections. To accomplish this, vaccine-induced immunity will need to extend beyond antibody responses and include robust DENV-specific T cell responses. In this context, a key question that remains to be answered is the extent to which cross-reactive T cells, particularly those induced by vaccines that do not encode the full proteome of all four DENVs (such as TAK-003 and Butantan-DV/TV003), can contribute to controlling viral replication and preventing severe disease in cases of breakthrough

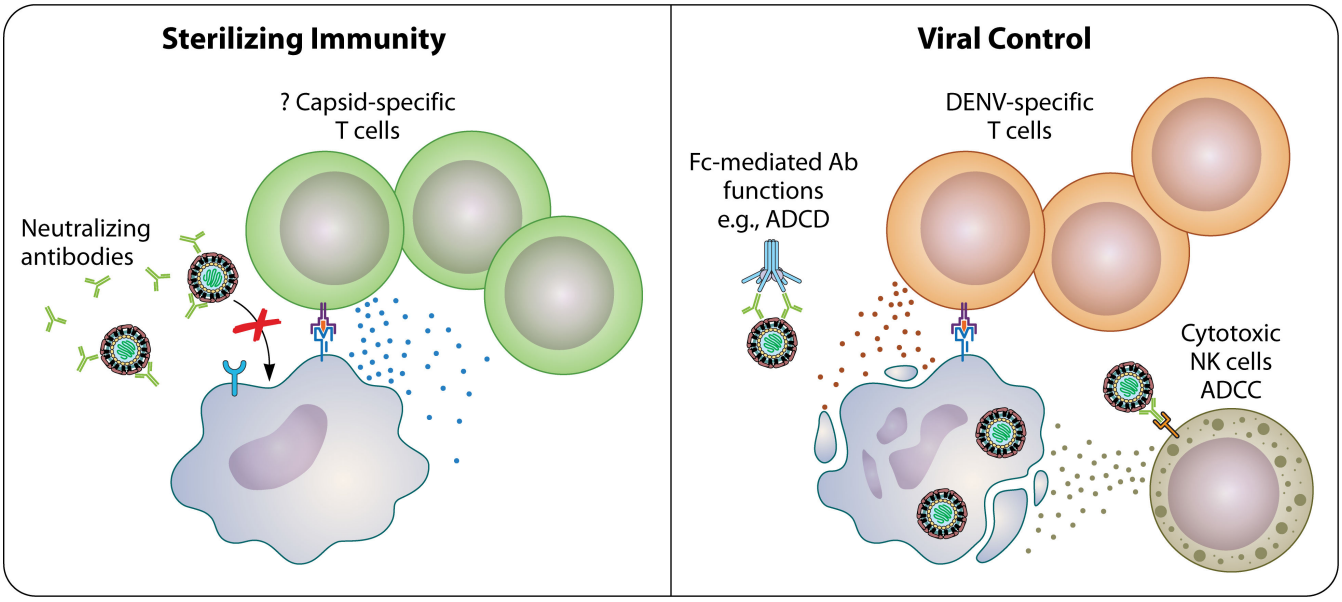


FIG 10 DENV correlates of protection. Although a definitive immune correlate protection against DENV infection has not been established, several components of the adaptive immune response likely contribute to protection in a coordinated manner. Sterilizing immunity is mediated by both type-specific and broadly cross-reactive neutralizing antibodies that prevent viral entry into host cells. T cells directed specifically against the C protein may also play a role in sterilizing immunity, although their contribution remains to be fully elucidated. In contrast, viral control in the absence of sterilizing immunity is mediated by cytolytic DENV-specific CD4 +and CD8+ T cells. Additionally, Fc-mediated antibody effector functions, including antibody-dependent complement deposition (ADCD) and antibody-dependent cellular cytotoxicity (ADCC) by cytotoxic natural killer (NK) cells, may also contribute to viral control.

infection. Addressing such a question may indeed require controlled human challenge studies to elucidate the role of vaccine-induced T cells and define CoP more precisely. In parallel with ongoing efforts to develop improved vaccine constructs, alternative strategies such as heterologous prime-boost regimens using currently licensed vaccines could be explored to broaden the immune response and enhance protective efficacy.

In conclusion, advancing dengue vaccine development will require a paradigm shift from a sole focus on nAbs toward a more holistic assessment of immune protection. This includes placing greater emphasis on T cell immunity and identifying composite immune correlates that predict protection against severe disease. Such an approach will be essential to guide rational vaccine design and deployment in dengue-endemic settings.

DENGUE HUMAN CHALLENGE STUDIES: AN ALTERNATIVE APPROACH TO ADDRESS KNOWLEDGE GAPS AND AID COUNTERMEASURE DEVELOPMENT

Given the challenges that remain in understanding dengue immunopathogenesis, and in advancing dengue therapeutic discovery and vaccine development, alternative approaches are needed to fill knowledge gaps and address questions on rare events or infection outcomes that are difficult to address in conventional trials or field studies. One such approach is dengue human challenge studies. Such studies have been used as far back as 1902 (420), although today, dengue human infection models (DHIMs) or controlled human infection models (CHIMs) are rigorously designed clinical research studies conducted under strict ethical and safety oversight. In these studies, healthy consenting adult volunteers are deliberately exposed to a well-characterized, attenuated or partially attenuated dengue virus strain under controlled conditions. DHIMs and CHIMs can overcome key limitations inherent in natural infection studies and field trials by enabling: (i) precise control over the timing of infection, facilitating collection of pre-infection and early acute-phase samples; (ii) use of standardized viral strains and inoculation doses to reduce variability; (iii) intensive longitudinal sampling to characterize viral kinetics, early host immune responses, and correlates of protection or risk; and (iv) smaller cohort sizes that allow for early stage evaluation and down-selection of vaccine and therapeutic candidates before proceeding to large-scale efficacy trials.

Two challenge models have been broadly established and are currently in use, both to study dengue immunopathogenesis, as well as to support dengue vaccine and therapeutic development (Table 2). These models differ in their choice of DENV strain, degree of viral attenuation and clinical phenotype, and serve distinct purposes as either disease or infection models.

The first model was pioneered by the Walter Reed Army Institute of Research (337) and now further developed by investigators at SUNY Upstate Medical University. This model utilizes under-attenuated DENV-1 (strain 45AZ5) and DENV-3 (strain CH53489) viruses that were initially designed as monovalent vaccine candidates but were later deemed too reactogenic for continued vaccine development (421, 422). Both strains have been shown to be safe, reliably infectious, and clinically informative in flavivirus seronegative adult volunteers. Challenge with either strain results in detectable viremia, fever, rash, and other clinical and laboratory features consistent with mild-to-moderate dengue-like illness, without progression to severe disease (337, 423–426). This model serves as a controlled *disease* model and has been employed both to study dengue pathogenesis and in early-phase vaccine trials to assess protective efficacy (337, 423, 426). Notably, this model was recently used to evaluate a tetravalent prime-boost vaccine regimen (PIV/LAV), which, rather than demonstrating protection, appeared to enhance infection, highlighting the value of DHIMs for down-selecting suboptimal candidates early in development (338). However, due to its capacity to induce symptomatic disease, the use of this model in dengue-seropositive individuals remains uncertain, given concerns about immune enhancement and the potential risk of severe disease.

In contrast, the second model, developed by Johns Hopkins University in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID), uses

TABLE 2 Key features of the two dengue human challenge models currently in use

	Disease model (WRAIR/SUNY Upstate)	Infection model (Johns Hopkins/NIAID)
DENV strains	DENV-1 (45AZ5) DENV-3 (CH53489)	rDEN2Δ30 rDEN3Δ30
Attenuation	Under-attenuated	Under-attenuated
Goal of model	To model symptomatic dengue fever	To model DENV infection (viremia ± mild clinical symptoms)
Volunteer profile	Flavivirus-seronegative healthy adults	Flavivirus-seronegative healthy adults
Challenge dose	DENV-1 (45AZ5): 3.25×10^3 PFU DENV-3 (CH53489): 0.7×10^3 PFU	rDEN2Δ30: 1.0×10^3 PFU rDEN3Δ30: 1.0×10^4 PFU
Peak viremia	DENV-1 (45AZ5): ~4.0 $\log_{10}$ PFU/mL DENV-3 (CH53489): ~3-7 $\log_{10}$ PFU/mL	rDEN2Δ30: ~2.5 $\log_{10}$ PFU/mL rDEN3Δ30: ~1.0 $\log_{10}$ PFU/mL
Clinical outcomes	Symptomatic dengue fever-like illness in a subset – Fever, rash, headache, leukopenia, thrombocytopenia, elevated liver enzymes	Mild illness in most – Rash (90%) – Fatigue, headache, and retro-orbital pain – Mild neutropenia and thrombocytopenia – Fever extremely rare
Level of risk	Moderate	Low
Applications	Study of dengue pathogenesis Evaluation of vaccines and therapeutics	Characterization of host response to DENV infection Evaluation of vaccines and therapeutics
Limitations	Secondary heterologous DENV challenge may be associated with increased risk of severe disease	Only suitable for evaluating antiviral efficacy in reducing viremia May overestimate vaccine/therapeutic efficacy in real-world settings

live-attenuated recombinant DENV-2 and DENV-3 viruses (rDEN2Δ30 and rDEN3Δ30, respectively) (427, 428). Originally developed as monovalent vaccine strains, these viruses have been repurposed for use in controlled infection studies due to their well-characterized attenuation profiles and favorable safety; 100% and 85% of individuals develop detectable low-level viremia when challenged with either rDEN2Δ30 or rDEN3Δ30, respectively, and almost all participants develop a characteristic rash (336, 429). Laboratory abnormalities, such as neutropenia and thrombocytopenia, are less frequent, and fever is extremely rare (336, 429). This model functions primarily as a controlled *infection* model and has been used to characterize the host response to DENV infection, in addition to being instrumental in the clinical development of the tetravalent live-attenuated vaccine TV003 (430–433). A limitation of this infection model is that it is likely only suitable for evaluating therapeutic efficacy when reduction in viremia is a meaningful endpoint. Moreover, the use of this model may overestimate the real-world efficacy of a vaccine or therapeutic intervention in preventing or mitigating disease. However, compared with a disease model, it offers potential advantages in terms of safety and ethical acceptability, particularly for use in dengue-seropositive individuals, including in studies involving heterologous DENV challenge.

Importantly, outcomes using both challenge models have to date only been reported in flavivirus seronegative individuals residing in dengue non-endemic settings. It remains to be determined whether the clinical and immunological outcomes observed in these studies will generalize to dengue-endemic settings, even among individuals without prior dengue exposure. Factors such as differing background immunity, host genetic differences, and environmental influences may modulate host responses and disease expression in ways not fully captured in non-endemic populations. Furthermore, in both challenge models, the virus is administered via subcutaneous injection, which does not replicate natural transmission by mosquito bite. Given that mosquito saliva has been shown to influence the kinetics and magnitude of DENV viremia (434), it remains uncertain whether the mode of virus delivery, for example, needle versus mosquito bite, may affect outcomes.

TABLE 3 Key questions and knowledge gaps in dengue

Topic	Questions
Virus-host interactions and pathogenesis	What virus-host interactions are critical for DENV pathogenesis?
	How do viral genetic variations mechanistically influence DENV fitness?
	How will climate change shape DENV evolution and transmission dynamics?
	What are the immunoprotective and immunopathological responses that, respectively, mediate protection against or progression to severe dengue?
	What is the impact of immunosenescence on the risk of severe disease in older adults?
Clinical management and therapeutics	How should older adults with dengue be clinically monitored and managed?
	What is the optimal fluid replacement strategy for adults with dengue?
	What are appropriate and meaningful endpoints for dengue therapeutic clinical trials?
Dengue vaccine development and application	Can a correlate of protection against dengue be defined?
	Does lack of vaccine efficacy necessarily translate to an increased risk of severe disease?
	How should dengue vaccination strategies adapt to changing population demographics and the increasing burden of disease in adults?

Overall, despite their limitations, human challenge models provide a valuable platform for addressing knowledge gaps in dengue immunology, the relative contribution of the different immune components in protecting against dengue, and evaluating new countermeasures.

CONCLUSION

The growing global public health burden of dengue calls for continued urgency into addressing gaps in both knowledge and countermeasure development against all four DENVs (Table 3). It is hoped that this review serves as a resource to help bridge these knowledge gaps, generate evidence-based management of dengue in older adults, spur the development of effective antivirals, and guide the safe deployment of dengue vaccines.

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